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THE TIME OF ACTION OF GENES, AND ITS BEARING ON SOME EVOLUTIONARY PROBLEMS

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ANY classification of variations must at present be provisional, largely because of the extremely limited sources of information which we possess. To take two simple examples, the white flowers on dark-stemmed races of *Primula sinensis* contain flavone. This gives a bright or faint yellow color with ammonia, according as the plant does or does not carry a gene B which modifies anthocyanin color when anthocyanin is present. If our vision extended far enough into the ultra-violet to reach the flavone absorption bands, it would be possible to distinguish these whites without using ammonia. In the same plant a gene R, apparently by causing an acidity of the cell sap greater than that found in rr plants, converts blue flowers into red, the anthocyanin acting as an indicator, but produces no visible effect in the absence of anthocyanin. A genetically minded caterpillar, which regarded the taste of the petals as more interesting than their color, would (one may suppose) readily detect the gene R in white-petalled plants, just as we classify cherries into sweet and sour by their taste. It is highly probable that many morphological differences between varieties are caused by chemical differences appearing earlier in the life-cycle, which could be detected by suitable methods, so that the stage in the life-cycle at which a character appears is necessarily a function of our means of observation. Indeed with a

G. Besides the above-mentioned genes in mosses, certain genes of the higher plants affect only gametes which carry them. Such are the genes which determine the type of polysaccharide in the endosperm of *Oryza* (Parnell, '21) and *Zea* (Brink and Abegg, '26). In each case the dominant form has ordinary starch, the recessive ("glutinous" and "waxy") contains a polysaccharide of different physical properties and giving a red color with iodine. The pollen grains contain the same polysaccharide as the endosperm in pure lines. But in heterozygous plants half the pollen grains contain each type of polysaccharide, and there can be little doubt that the type is determined by the gene carried by the pollen grain concerned. Parnell found that the immature pollen grains immediately after meiosis contain no starch. This develops gradually, under the influence of the particular gene carried by the grain. Similarly, in some but not all species, a pollen grain borne by a zygote with irregular meiosis attains to a size roughly proportional to the numbers of its chromosomes.

The genes determining membership of an intrasterile group in such plants as *Nicotiana* (East and Mangelsdorf, '26) fall into this class, and also into class Z4. Genes falling into class G only are indeed rather rare, but include the gene Ga for pollen tube growth-rate in *Zea Mays* (Mangelsdorf and Jones, '27). I do not know of any single gene in class G which affects megaspore characters. It is not yet clear whether the effects of Renner's "complexes" in *Oenothera,* which influence both microspores and megaspores, are due to single genes or gene groups. It is a noteworthy fact that no genes of this class are known in animals. Muller and Settles ('27) showed that the viability of the spermatozoa of *Drosophila* did not depend on genes either in the X-chromosome or in a section of the second chromosome which is essential for the life of the zygote. It is, moreover, known that apyrene spermatozoa may be fully motile, and it seems unlikely that the chromatin of a

spermatozoon has any relation other than a purely mechanical one to its other constituents. Doubtless this difference between higher animals and plants is due to the fact that in the latter the gamete represents a suppressed generation, which still preserves its own specific physiology. The difference is important from the evolutionary point of view because it implies that certation between gametes, which is an important type of natural selection in plants, is relatively unimportant in animals. It is also probably one reason why no animal is known whose genetics correspond to those of *Oenothera*.

E. Numerous genes are known affecting the endosperm in *Zea Mays*. Some, *e.g.*, "waxy," also fall into class G. Others, such as the gene for carotene (provitamin A) formation, are not known to act except in the endosperm. These genes have, of course, no analogies in animals, though possibly genes may be found controlling the structure and function of the tissue formed from the polar bodies in such forms as *Litomastix*.

MZ. This is the least studied group of genes with which we have to deal. Metaxenia, *i.e.*, difference between the influence exerted by different kinds of pollen on diploid maternal tissue, is quite a well-known phenomenon. Thus Crane and Lawrence ('29) showed that in *Prunus* and *Rubus* drupes or drupels generally require the formation of an embryo, and abort if it perishes early enough. But the differences in embryonic development thus influencing the maternal tissue were due, not to single genes, but to abnormalities involving whole, chromosomes. Nixon ('28) and Swingle ('28) showed that in *Phoenix dactylifera* the pollen had a considerablç influence on the fruit, fruit size and seed size being core related. Here the effect is more probably to be ascribı as to genes, though no analysis has been made. Metaxeı is of course only part of the action of genes of group) If the mother is heterozygous for genes of this group shall find variation in fruit size due to this fact evı pollen of a homozygote is used. Tschermak ('31) a bibliography of MZ and Z action in plants.

There can be little doubt that (although no MZ genes are known in animals) genes of the MZ type have been of extreme importance in mammalian evolution. The foetus has a great influence on the mother, which must have been evolved by successive steps, and until such genes have been observed in mammals a large chapter in their evolution will remain obscure. Thus the placenta is known to contain hormones capable of acting on the mother, but nothing is known as to their variation. Nor do we know whether genetic factors in the foetus may not transform it into a chorion-epithelioma which kills the mother.

Z1. This group includes a number of well-known plant genes, such as Mendel's gene-pairs for round and wrinkled, yellow and green, cotyledons in *Pisum*. Some characters show at this stage, and are confined to it (*e.g.,* round and wrinkled). Others persist through life (*e.g.,* the sap color gene in *Matthiola,* Frost, '28). In animals the situation is not so good. Some lethal genes are known which act at this stage, but they may be regarded as acting throughout the rest of the life-cycle, as adults bearing them are conspicuous by their non-existence. An example of a gene acting at this stage is Toyama's ('13) recessive gene for crimson serosa color and reddish larval head color in *Bombyx mori*. The importance of purely embryonic variation in evolution is clear when we consider the extreme divergence of the early embryonic forms of species in which the adults are fairly similar, such as *Lepus* and *Cavia*. We do not know if this is due to Z1 or DZ genes, or possibly to extranuclear plasmons. The study of genes whose action is confined to the Z1 stage in higher animals is an important but difficult field for future research. A beginning has been made by Gregory and Castle ('31). They showed that the eggs of a race of large rabbits, the number of blastomeres at 40 hours after mating was 9.94 ± .24, in a small race 8.29 ± .19. In hybrids derived from the of a large female and spermatozoa of a small male

the number was $8.44 \pm .40$. In this case it is clear that Z1 genes carried by the spermatozoon had an important influence on cleavage rate, which was not mainly determined by DZ genes.

Z2. Apart from lethals, and genes for leaf shape and chlorophyll which show at this stage and persist through life, genes of this class are not very common in the higher plants, where seedlings are on the whole more uniform than adults. There is a little evidence for heritable variation in cotyledon shape not due to genes whose action is apparent in the adult, in *Primula sinensis*.

In animals such as holometabolous insects with a well-marked larval stage, differences confined to this stage are not uncommon, *e.g.*, several genes for larval color in *Lymantria monacha* (Goldschmidt, '27).

Z3. This class includes the majority of genes studied in animals, and all those in plants which affect the leaf and stem. These latter may or may not influence the flower.

Z4. This group includes the genes for flower character in plants; and a good many animal characters, *e.g.*, intersexuality, milk yield and egg yield fall into it. Perhaps the genes governing feather characters in birds which depend for their expression on the sex hormones (*e.g.*, henny feathers in poultry) should properly be included here.

Z5. This is perhaps merely a sub-group of Z4, but assumes a special importance in plant genetics, owing to the importance of fruits. Genes of class Z5 interact with E, Z1, MZ, and possibly DZ genes. Thus the shape of peas is conditioned by Z5 genes such as "indent," which acts in the maternal seed coat, and to a greater extent by Z1 nes, such as "wrinkled," which act on the cotyledon; the pod character is mainly determined by Z5 genes. the other hand, in the cherry and date Z1 genes are ively unimportant except in so far as they also fall roup MZ.

nimals Z5 genes are not so important, but for the symmetry such characters as those of the egg-

shell and albumen, which depend on secretions of the oviduct, may be included here. In the evolution of the mammal a critical part was doubtless played by Z5 genes which interact with MZ genes in the foetus to determine a suitable response of the uterus to its contents. We have not, of course, the faintest idea which group played the principal part in the evolutionary process.

DG. Delayed gametic characters are not uncommon in plants. Sometimes a gene is only known to act on gametes, *e.g.*, the gene for round as opposed to oblong pollen in *Lathyrus odoratus*. Sometimes it acts at other periods in the life-cycle. *E.g.*, the gene S in *Primula sinensis* produces a short style and long anthers, and also large pollen grains. Usually these genes seem to act wholly or mainly on the pollen, but the "polymitotic" gene in maize (Beadle, '30) causes extra nuclear divisions in gametes of both genders.

It must be emphasized that these genes act on all the gametes of zygotes carrying them, whether or not they are present in the gametes concerned. In some cases, *e.g.*, where size or shape of the grains is affected, their action does not continue in grains which do not contain them. But the normal allelomorph of the polymitotic gene suppresses supernumerary divisions of gametes borne by heterozygotic plants, even when those gametes carry the polymitotic gene. It clearly has a lasting influence on the surrounding cytoplasm.

Lesley and Frost ('27) describe a gene L in *Matthiola incana* which is on the borderline between Z4 and DG. LL and Ll plants have normal meiosis, with short chromosomes during meiosis. ll plants have long chromosomes during this period. Philp and Husk ('31) regard the extra length as due to a delay in onset of prophase contraction. This gene also has a effect, in that ll plants produce an unusually large portion of gametes with chromosomal irregula Chromosomes may be lost or fragmented, or chromosomes may enter gametes, giving rise to tri

If, therefore, the cytology of ll plants were not known, l might be regarded as a recessive DG gene for "bad pollen."

No cases are known with certainty of DG genes in animals, though it should be possible to detect the action of some DZ genes in the unfertilized egg. Thus it would almost certainly be possible to ascertain the color of silk-worm egg-yolks in the ovary before fertilization. Since, as we saw above, it appears that genes do not function in spermatozoa, it is probable that the main differences between spermatozoa of different species are determined by genes of this class.

DZ. Genes of this group do not appear to be known with certainty in plants, though Miss Pellew informs me that the size of adult *Pisum* plants depends to a considerable extent on seed size determined by the mother and not the father. However, in this species inheritable variation in seed size occurs among peas in the same pod, *i.e.*, some of the genes determining size fall into group Z1. We have then not only to reckon with the fact that, besides environmental effects, genes of both these classes determine seed size, but with the possibility that the same gene may fall into both groups Z1 and DZ. If this is so the genetics of seed size will prove exceedingly complicated.

In animals DZ genes are well known. The classical case in *Bombyx mori* was described by Toyama ('13) as "maternal inheritance." This phrase might be taken to cover extra-nuclear inheritance, in which all inheritance takes place through females only. The tendency of modern genetics is to describe genetical facts in terms of gene action rather than heredity. So possibly "delayed gene action" is a more lucid phrase than "maternal inheritance." Some of Toyama's characters, such as spindle-shape and roughness of egg-shell, are Z5 characters due to a purely maternal structure. Others, such as yellowness of yolk, associated with yellow blood in the mother, are presumably DG. Others, however, such as

blueness, are due to pigment formed in the serosa, a zygotic structure, but do not seem to be at all influenced by the father. Along with these occur Z1 genes such as that for crimson eggs.

Since then Boycott, Diver, Garstang and Turner ('31), have shown that dextrality and sinistrality in *Limnaea peregra* are DZ characters, presumably depending on the structure of the unfertilized egg. Redfield ('26) discovered a lethal DZ gene in the second chromosome of *Drosophila melanogaster* affecting female embryos much more than males.

Experiments on interspecific hybridization in fish and echinoderms show that the rate and type of cleavage is mainly conditioned by the cytoplasm. If so it is controlled either by plasmons (characters inherited outside the nucleus) or by DZ genes. The evidence from species crosses yielding fertile hybrids shows that on the whole plasmons are far less important than genes in determining early development, not only in species where the cytoplasm of the egg is obviously differentiated, as in molluscs, but also where this is not the case, as in echinoderms. In mammals and other viviparous animals some maternal genes doubtless have a DZ action on the embryo through the uterus, and it would be hard to distinguish them from DZ genes of the normal type.

The range in time covered by a single gene is a matter of some interest, though data are scanty, because only very striking differences in the gamete and embryo are noticed at all. Thus C in *Pisum* is necessary for anthocyanin formation in the axils, color in the petals, the pods, and also in the seed-coat, *i.e.*, ranges from Z3 to Z5. But its detection depends on the presence of other genes. It does not for example affect the axil unless a special gene is present, or the seed-coat unless one of several genes is present. Perhaps a suitable chemical technique might detect its presence at earlier or later stages.

Frost ('28) gives an example of a gene acting over more than a life-cycle, so that a Z1 gene of one generation

interacts with a DZ gene of its predecessor. BB and Bb *Matthiola incana* (provided the color genes C and R and a plastid gene W are present) have purple flowers, bb being red. BB plants have black seeds, and bb brown. On the whole a Bb plant selfed gives 3 black (BB and Bb) to 1 brown (bb) seeded. But among the black seeds a few give red-flowered (bb) plants. Clearly the main action of B is to blacken embryos carrying it. But it may have a slighter effect in blackening bb embryos borne by zygotes carrying it. Fortunately, B can be scored unambiguously on flower color, but if it only affected seed color the genetic analysis would be very difficult. We must be prepared to find characters presenting this difficulty. Hogben ('31) thinks that in man blue sclerotics are due to a dominant gene of this type.

From the point of view of this paper certain evolutionary problems appear in a rather new light. As a general rule the embryos of related organisms are more alike than the adults. This merely means that the genes which determine interspecific differences mostly come into action rather late in the life cycle. The gill-clefts of embryo fish persist throughout life. Those of *Amniota* are transformed or disappear. The genes responsible for this difference must be present throughout the life-cycle, but only come into action during the stage Z2. The opposite is occasionally the case. The adult forms of many animals resemble one another more than the larval forms. Examples are the larva of *Unio* as compared with that of related salt-water mussels, the larvae of *Culex, Chironomus* and *Corethra,* and so on. These presumably differ by genes whose action is mainly confined to stage Z2.

There has been a common tendency in evolution for development to accelerate, *i.e.,* for certain characters to appear progressively earlier in the life cycle. Thus in the *Ammonoidea* complicated types of suture line first appear in the adult, and then progressively earlier in development. This presumably means that the time of

first action of certain genes has tended to be pushed back from stage Z4 or Z3 to Z2 and Z1. Although the genes may have been somewhat modified in the course of ages, this seems a simpler hypothesis than that quite new genes arose which produced an effect similar to that of the ancestral genes, but at an earlier stage.

Another common tendency has been a retardation of certain characters relative to the life-cycle, so that originally embryonic characters persist in the adult. This is known as neoteny or sometimes paedogenesis. In a neotenic animal such as man many adult ancestral characters, *e.g.*, the straightening out of the cranial flexure, do not occur. Presumably the genes causing cranial flexure, which in most mammals are confined in their action to stages Z1 and Z2, act for longer in man. Or alternatively various genes which act in stage Z3 of most mammals have either been lost or never come into action in man.

These are of course well-known examples. But so far the existence of G, DG and DZ genes has been forgotten in their discussion. In acceleration Z3 genes come to act in stage Z2, in neoteny the opposite process occurs. The result is then the sudden appearance in an adult form of new characters, which have evolved in ancestral embryos. De Beer ('30) to whose book I am indebted throughout this paper, describes this process as clandestine evolution. I think that the same processes may have an even wider scope. G genes may come to act in stage Z1 and conversely. Z3 and Z4 genes may come to act in stages DG and DZ and conversely.

It is possible that some of the more mysterious of evolutionary phenomena may be explained in this way. Very early *Ammonoidea* such as the Devonian *Bactrites* were straight, and the inner whorls of its contemporary *Mimoceras* did not meet. These primitive forms were replaced by organisms with normally coiled shells. But in later periods of degeneration the shells of a number of genera were coiled during the early part of the life

cycle, but later uncoiled. Such were the Triassic *Choris-toceras* and *Rhabdoceras,* the Cretaceous *Lytoceras* and *Bactrites.* The general tendency in Ammonoid evolution had been to push back the action of genes from Z3 to Z2, and so on. I suggest that the gerontic straightness of degenerate forms was due to the pushing back of DZ genes governing embryonic development into the Z4 and Z3 stages. The adult *Bactrites* formed an uncoiled shell for the same reason that its ancestors formed a straight protoconch. The same argument could be applied to other cases where, after a long evolutionary history of acceleration, embryonic characters appear in the later stage of life-history.

The opposite process of caenogenesis may also perhaps occur in three ways, of which only the first has apparently been recognized. New Z1 or Z2 genes may appear. New DZ genes may appear. And finally Z3, Z4 or DG genes may come to act in stage DZ. We should expect the last to happen in connection with a general process of retardation. To take an example, Garstang ('28) has suggested that gastropod asymmetry began as a larval adaptation. We know that it is largely conditioned by DZ genes. These may have first appeared as such. But they may also have at first been Z3 or Z4 genes responsible for relatively trivial asymmetry in the adult or its cells, whose action was retarded in relation to the life-cycle, and thus came to produce larval asymmetry.

Whereas the change in time of action of a gene from Z2 to Z3, or conversely, presumably has effects which can at least be roughly predicted, this is not in general true for the changes from Z3 to DG or DZ, and conversely. Such changes are likely to lead to violent evolutionary novelties. A G or DG gene responsible for a lipase or protease in the spermatozoon might render uterine implantation possible for extending its action to cover stage Z1 or DZ. A DZ gene for rapid growth might cause malignancy if it came to act in stage Z4, and so on. The possibilities of clandestine evolution have probably been underestimated.

It may be objected that the above point of view is some-
what superficial. A DZ gene for shell straightness in an
Ammonite, if its action were accelerated, would come into
action of some kind, it might be urged, in the oocytes of
the ovary, but not in the somatic cells responsible for shell
form. I doubt if this objection can stand. Presumably
during the life-cycle the protoplasm of the cells in the
germ-track passes through a cycle of chemically definable
stages, each being associated with a particular pattern
of metabolism, and each being to a large extent the cause
of the next. It is, however, possible, within certain
limits, to alter the timing of one event relative to others,
just as the time of ignition in an internal combustion
engine may be altered relatively to the piston stroke.
The cells lying off the germ-track seem to remain fixed
for a long time in one stage of the cycle. If, however,
other processes were accelerated in relation to meiosis,
this might mean that not only oocytes, but somatic cells,
passed into a chemical condition which had hitherto only
been found in the embryo.

Acceleration or retardation can occur in two different
ways. Thus in the case of acceleration the appearance or
disappearance of one or many characters may occur at
an earlier stage in the life-cycle in a descendant than in
an ancestor. If only one or a few characters are con-
cerned it is reasonable to suppose that the essential event
was the acceleration of the action of the genes determin-
ing them (*e.g.,* the heart in birds or the horns in *Titano-
theria*). But where the acceleration was general, as in
certain lines of *Ammonoidea,* it is simpler to regard the
process at work as a retardation of maturity in relation
to other events. Such a retardation would of course
allow DG and DZ genes to come into action before instead
of after meiosis.

The reduction to vestiges of organs present in the adult
ancestor and retained in the embryo (*e.g.,* notochord or
pronephros) may often be due to acceleration. In some
fish the notochord only ceases growing with the body as
a whole. In other fish, and all *Amniota,* it ceases to grow

(apart from cases of chordoma) in an embryonic stage. This may be due to the presence of inhibitory genes, or merely to a pushing backwards of the time of activity of some gene or genes involved in notochord growth. In view of the large number of other vestigial organs which have shared the notochord's fate, the latter hypothesis is perhaps simpler.

So with the retardation of the appearance or disappearance of characters. This is most usually part of the general phenomenon of neoteny, which can be regarded as due to accelerated maturity in some cases (paedogenesis) and general retardation of structural development in relation to maturity in others (*e.g.*, man). It probably occurs also for individual characters in the absence of any general neoteny, but I do not know of any clear examples of this.

In the evolutionary speculations of the last century hypermorphosis played an important part. The descendant was supposed to go through all the stages of its ancestor, and a final stage in addition. As de Beer ('30) and others have pointed out, many of the alleged examples of this process can be equally well explained by deviation. From the genetic point of view such a process involves one of two events. Either a large number of new genes must come into being which act in stage Z3 or Z4, and simultaneously maturity must be delayed; or, by a general acceleration, DG and DZ genes must come to act in stages Z3 or Z4. The former process is a complex one needing many simultaneous adjustments. It is difficult to believe that it has occurred very often. The latter would be simpler, but might lead to racial "second childhood."

Underlying all the above speculations is the tacit assumption that the same process may accelerate or retard the appearance or disappearance of the activity of a large number of genes at once. I believe that this is justifiable. In many cases genes seem to act, not directly, but by producing enzymes. Whether these latter can be effective depends on circumstances. For example, catalase is es-

sential for the well-being of bacteria in presence of oxygen, but in its absence forms with and without catalase may do equally well. Koller ('30) found that rabbits with the gene E^D produced more (or a more resistant) certain lines of *Ammonoidea*, it is simpler to regard the dioxyphenylalanine oxidase than EE rabbits. But the question whether or not this gene will have any visible effect depends on the presence or otherwise of the inhibitor G, which produces little effect on rabbits with E^D. Now enzymes depend particularly on the pH and salt content of their medium. The latter at any rate often varies greatly during development. The growing tadpole absorbs proportionately much more water than salts from its environment. This process will affect a whole group of enzymes (*e.g.*, amylase, fumarase and catalase) and have very little effect on others. Thus a change in permeability due to a single gene would affect the time of action of many others. The same substance may be required for several quite separate processes, *e.g.*, glutathione for oxidation (Hopkins and Elliott, '31) proteolysis (Waldschmidt-Leitz, Purr, and Balls, '30), and starch hydrolysis (Pringsheim, Borchardt, and Hupfer, '31). So the biochemical effects of one gene may accelerate or retard the action of a whole group of others.

Equally important is the fact that at least in vertebrates the development of many characters is conditioned by hormones, *e.g.*, thyroxin and various gonadic secretions. So several processes exist by which the time of action of a number of genes could be accelerated or retarded simultaneously.

The gradual acceleration or retardation of a number of genes will lead to orthogenetic evolution. In many cases, as Goldschmidt ('27) has pointed out, the sooner a gene starts work, the more it can do. So that selection for a character will not merely cause the spread through a population of genes directly causing that character, but of genes accelerating their action. These latter will probably accelerate the action of other genes as well, leading to apparently useless evolution.

Two types of selection can be mentioned which probably cause general acceleration and retardation, respectively. When a number of embryos or larvae are competing in a limited area, *e.g.,* embryos in a mouse uterus, or seedlings from seeds dropped by the same tree, rapid growth will commonly be of great selective value, and the slower growing individuals will be weeded out. There will be a tendency to cut short the period of intense competition, and to push back the first appearance of characters as early as possible. So Z2 genes will begin to act in stage Z1, Z3 in Z2 and so on. This will react on the adult form both by giving certain genes longer to act, and possibly by accelerating the action of DG or DZ genes.

On the other hand, where a larva or embryo is well adapted to its surroundings, and can go on growing in relatively slight danger, there will be a tendency to prolong the embryonic phase. Examples may be found in the human embryo, which rarely suffers from twin competition, and in a savage country may well be safer than a new-born baby, or the aquatic larvae of many insects, which are well adapted to their surroundings, and do not generally compete directly with one another. In such cases the appearance of adult characters may be delayed. Z2 genes will not begin to act till stage Z3, and so on. There will be a tendency to neoteny, and possibly a retardation of Z3 and Z4 genes to act in stages DG or DZ. This can of course be counterbalanced by intercalating, as in the holometabolous insect, a period of catastrophic metamorphosis, during which genes act with very great speed. This again may lead to unpredictable results. Genes particularly adapted to the chemical environment of the chrysalis will assume particular importance. Others will cease to have selective value. As the metamorphosis becomes more and more pronounced there will probably be a tendency to select, on the one hand, genes acting only at this time, on the other, genes whose action abruptly ceases then. The more the time of action of genes can be limited, the more the possibility of combining adaptation in the larva and adult.

In general the more limited is the period of action of a gene, the more unalloyed will be its benefits if it is useful at a certain period.   There is no reason to suppose that the same type of polysaccharide is desirable in pollen and endosperm in maize, but so long as one gene controls both, the adaptation of the endosperm will be subordinated to that of the pollen grain, or conversely.   The greater the difference in cell chemistry at different stages of the life-cycle the greater should be the possibility of limiting gene action.   It would therefore seem that within limits natural selection will tend to make life-cycles more and more variegated, as every increase in complexity will increase the possibility of fixing the time of action of genes.   Clearly there are limits to the process, but it may be a partial explanation of some of the apparently useless complexities of biology, such as the tendency of parasites to live in widely different hosts. We must consider the possibility that a liver-fluke is well adapted to its very different environments just because on changing from a diet of *Limnaea* to a diet of *Ovis* at a higher temperature, the chemical conditions in it are so changed as to allow a new set of genes to come into action, and it can thus possess two sets of genes, one acting in each host, and each capable of evolving in almost complete independence of the other.

Another way of fixing the time of action of genes is by elimination of part of the chromatin in somatic cells, as in *Ascaris* and *Miastor*.   This method has, however, rarely been adopted.   It must involve the localization together of all genes whose action is limited to an early stage of development, and are not essential later on.   In view of the lack of correlation between the location of genes and their function, it is surprising that such elimination ever occurs, and intelligible that it is rare, although it is the mechanism which one would obviously adopt in designing a "synthetic animal."

We see, then, that the time of action of genes not only merits further study by the geneticist, but is essential for a detailed discussion of evolution.

Summary

(1) Genes can be classified according to their time of action. Not only can they act in diploid zygotes, endosperms or gametes carrying them, but their action can be manifested in the following organisms which do not carry them: mothers of zygotes carrying them, and gametes or embryos borne by zygotes carrying them. Their time of action is therefore distributed over more than one life-cycle.

(2) It is contended that change in the time of action of genes has been an important factor in evolution, and that some cases of orthogenesis, including degeneration, can be explained by it.

(3) In an organism undergoing metamorphosis the adaptive efficiency of a gene depends on the limitation in time of its action.

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THE CONTROL OF THE CHROMOSOMES BY THE GENOTYPE AND ITS BEARING ON SOME EVOLUTIONARY PROBLEMS

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I. INDUCTIVE

THE primary postulate of genetics is that the properties of living organisms are the resultant product of the interaction of "genotype" and "environment," and on this assumption the study of their inheritance, variation and adaptation can be consistently developed. But it is not true of the properties of their chromosomes. The chromosomes have, it is believed, an individuality, a permanent structure, which can not be subject to the same control as that to which other organs are subject. This conclusion is forced on us from four directions. First, the original observations of Boveri on the continuity in the existence of chromosomes are supported by the great bulk of systematic observations: their form and number in each cell-generation are constant and their behavior is consequently predictable. Secondly, where changes are found to occur under experimental conditions they can nearly always be shown to arise from changes in the potentially permanent structure of the chromosome. These do not result from changes in genetic conditions but rather as accidents and actually themselves determine genetic changes (for predictions based on this assumption are regularly verified). Thirdly, the continual strengthening of the chromosome theory of heredity necessarily strengthens the belief in the permanence of the chromosomes, since heredity is merely a manifestation of permanence. Finally, the notion that there is something absolute about the properties of the chromosomes and that the relation between these properties and the genetic properties of the organism is therefore a

simple and direct one has been widely used as a basis of deduction and has therefore acquired the prestige of dogma.

But the permanent structure is internal to the chromosomes. And the question arises: is not a considerable variation in external form and behavior, such as would be genetically determined, compatible with strict permanence of internal structure? Evidence is beginning to show that such variation occurs. But the strength of the prevailing doctrine has often prevented the proper inference being drawn, and the isolated character of the observations has prevented their wider significance being seen. It will therefore be worth while to recall these observations and consider their meaning.

Our problem is to find out (i) to what extent changes in the properties of a chromosome are due to independent changes in the affected particles (*i.e.*, in permanent structure), (ii) to what extent they are due to the action of the chromosomes as a whole, the genotype, on their constituent parts and (iii) whether any third factor must be assumed. It must be borne in mind that, although the action of the genotype is customarily determined by observing the differences between two genotypes which are due to changes in certain of their constituent parts, a genotypic change will probably not be specific to the part affected. A structural change on the other hand will always be specific. Moreover, it will occur under two kinds of conditions, which can already be defined with moderate precision, *viz.*: (i) at random with regard to the position and type of change, whether fragmentation, translocation or other kind, and usually at any part of the life cycle but perhaps chiefly at meiosis. (ii) At the prophase of meiosis as a result of crossing-over between homologous parts of chromosomes which are associated with dissimilar parts. The first may be described as *primary,* the second as *secondary* structural changes since they can only arise from the occurrence of the first.

It follows therefore that the essential distinction between the results of a change in structure and in genotype is that the one is confined to the points at which it occurs, while the other is not so confined and is likely to be general in effect. We may then expect a difficulty to arise in applying this distinction when the organisms whose chromosome form and behavior are being compared are so remotely related that numerous structural differences may be supposed to have arisen between them. But when they are closely related, even though conditions of inheritance are not understood, we may hope to draw an unequivocal conclusion.

The evidence may therefore be classified, according to the stringency of the inference to be based on it, in five different categories, as follows:

(a) The difference is characteristic of races and is inherited as a Mendelian character (*e.g.*, chromosome contraction at meiosis in *Matthiola* or failure of meiosis in *Zea*).

(b) The difference arises through mutation in a known individual (*e.g.*, chromosome size in *Tradescantia* or parthenogenesis in *Rhabditis*).

(c) The difference is seen between the chromosomes of a hybrid and the same chromosomes in its parents (*e.g.*, chromosome size, length and constriction in *Crepis* and *Vicia*).

(d) The difference occurs between different stages in the development or parts of the body of an individual (*e.g.*, facultative parthenogenesis in *Allium odorum*, chromosome diminution in *Ascaris*) or in the life cycle of a species (*e.g.*, cyclic parthenogenesis in Aphides) while related species are constant in one form or the other.

(e) The difference occurs between related races, species or larger groups (*e.g.*, chromosome contraction in *Phragmatobia* and *Crepis*, compound chromosome formation and diminution in *Asçaris*).

While there can be little doubt of the cogency of the first three types of evidence, the last two may be thought insufficient. The type of inference is a rather new one although it has been used by Haldane ('31a) in analyzing variation in other organs from a similar point of view. The question, however, is simply this: can the differences involved be explained on an analogy by the recognized alternative of structural change? In my opinion, in the cases I am going to consider, they can not.

The following are examples of differences attributable to the subordination of the chromosomes to the geno-type. The first four and the last are concerned equally with mitosis and meiosis, the rest are peculiar to meiosis.

(i) *Chromosome size.* (a) A triploid plant, a presumed hybrid between tetraploid *Tradescantia virginiana* with large chromosomes and a diploid species with chromosomes about one fifth the bulk (but corresponding in form) had chromosomes corresponding in bulk to the larger-chromosome parent. One bud on this plant had chromosomes one fifth this size at the pollen grain mitosis. Its resting nuclei were approximately one third the size. It was therefore concluded that the change was due to a genetic mutation determining the bulk of chromosomes and nucleus and that such a mutation distinguished the parent species (Darlington, '29c and unpublished). (b) A difference in the size of the autosomes in male and female *Sphaerocarpus* is perhaps due to a less pronounced change of the same kind (Lorbeer, '30). (c) In the hybrid *Vicia sativa* × *Vicia angustifolia* (Schweschnikowa, '29a) the chromosomes are smaller at mitosis and meiosis than in the parents. This difference must also be regarded as a genetic property. It is important to notice that this variation is not attended with any significant change in cell size.

Whether the changes of size genotypically determined are due to a multiplication of the specific hereditary molecules or to a greater dispersion of them is a question that need not be considered here.

(ii) *Chromosome Contraction.* (a) In *Phragmatobia fuliginosa* (Seiler, '25) two races occur with different degrees of linear contraction (lateral expansion) at metaphase of mitosis (maximum contraction occurs in both forms at meiosis). The degree of contraction is constant throughout the complement and in different nuclei in the higher organisms generally. The diameter of the chromatids at metaphase is a specific character. When, therefore, it is found as a varietal character and no difference can be found in any other respect between the varieties it can be regarded as genetically determined.

(b) Similarly, in *Melandrium album,* Breslawetz ('29) found two individuals with chromosomes contracted to one third their normal length, as though at meiosis. This property was constant and therefore genotypically determined.

(c) It may be concluded that a difference of this kind distinguishes *Crepis capillaris* and *C. neglecta,* for the chromosomes of the first are longer and of the second shorter in the hybrid between them than in the parental species (Navashin, '31b).

(d) Clausen ('31c) finds a reduced longitudinal contraction of the chromosomes at meiosis in an F_3 segregate from the cross *Viola tricolor* $\times$ *V. Orphanidis.* This must also be regarded as a genetically determined reduction of contraction, probably characteristic of mitosis, since it is seen at the second division (*cf. Matthiola*).

(iii) *Chromosome Constriction.* In various first generation *Crepis* hybrids a trabant, *i.e.,* a small distal part of a chromosome separated by a long constriction from the main body, characteristic of a parental chromosome was found to be fused with the main body of the chromosome. Here, although the difference is single, as though produced by structural change, the structure of chromosomes must be the same in a hybrid and in the parent and the change must therefore be genotypic. This means therefore that the genotypes of parent and hybrid (as in the *Vicia* case above) differed in their reaction to the con-

strictions of chromosomes (Navashin, '26, Hollingshead, '30b). The disappearance of the constriction is probably a symptom of increased longitudinal contraction of the chromosome.

(iv) *Chromosome Aggregation and Diminution.* The chromosomes in somatic cells of *Ascaris megalocephala* undergo the well-known changes of fragmentation and diminution. Comparison with related species shows that the fragmentation is probably a reversal to an ancestral condition. The properties by which the separate elements are held together permanently in the germ line and by which parts of them are lost in the body cells, must therefore be genetic. This view is strengthened by the observation by Seiler and Haniel ('21) of reversible fusion at a particular stage of the life cycle in the male of *Lymantria monacha.* The temporary fusion observed in this species has apparently become permanent in the related species *Phragmatobia fuliginosa.* The property of diminution in *Ascaris* and *Miastor* is similarly a genetic-developmental reaction.

(v) *Precocity of Meiosis.* In *Matthiola incana* a race occurs with chromosomes less contracted at meiosis than in the normal type. They look more like ordinary mitotic chromosomes, and they are liable to be irregular in pairing (Lesley and Frost, '27, Philp and Huskins, '31). This property has been attributed to a partial failure of precocity in the prophase which is supposed to determine the pairing of chromosomes at pachytene and the characteristic exaggerated contraction at the metaphase of meiosis (Darlington, '31b). It is characteristic of the metaphase of the first division; it is scarcely appreciable at later stages and absent in ordinary mitosis. This distinguishes it from the abnormality in *Viola.* Since the property behaves as a Mendelian recessive, there can be no doubt of its genetic basis.

The local suppression of meiosis in *Allium odorum* (Modilewski, '30), its cyclic suppression in *Phylloxera* (Morgan, '15) and other parthenogenetic organisms, and exceptional suppression in individuals of some species

(*e.g.*, *Ascaris megalocephala bivalens*, Gcinitz, '15) are probably due to the complete failure of pachytene pairing and the replacement of meiosis by mitosis. They may be taken to represent an exaggeration of the effect observed in *Matthiola*. They are certainly determined genotypically and not structurally, *i.e.*, by hybridity. It may be supposed that these genotypic effects approximately reverse the process by which meiosis originally arose from mitosis by a too-early beginning of the prophase (*v. infra*).

(vi) *Chiasma Formation.* Clones of *Fritillaria imperialis* occur with differences in the frequency with which chiasmata are formed in the paired chromosomes at the diplotene stage of meiosis (Darlington, '30c). The difference is characteristic of all the chromosomes and of all the bulbs of the clone; there is therefore no reason for assuming hybridity such as would reduce the possibility of forming chiasmata in one clone, or for assuming environmental differences such as might modify the frequency, and the difference can be attributed only to genetic factors.

The suppression of the pairing of chromosomes at meiosis in *Zea Mays* (Beadle, '30) since it does not affect the linear contraction of the chromosome, is perhaps due simply to a complete suppression of chiasma formation and not to suppression of pachytene pairing. The same is true of the failure of pairing in the male-sterile *Viola Orphanidis* (Clausen, '30) and in the hybrid *V. nana* × *V. arvensis* (Clausen, '31). The maize abnormality is determined, like the reduction and suppression of crossing-over in *Drosophila* (Gowen, '28; Bridges, '29) by a single recessive factor. On the chiasmatype hypothesis, differences in crossing-over frequency and distribution are analogous to similar variations in crossing-over. The suppression of chiasma-formation is probably an alternative means to failures of pachytene pairing of securing failure of reduction in parthenogenesis (*cf.* Beadle, '30).

(vii) *Distribution of Chiasmata.* In *Mecostethus gracilis* and *Fritillaria Meleagris* the chiasmata are localized in the neighborhood of the spindle attachment (McClung, '14, '28; Janssens '24; Newton and Darlington, '30). This is probably to be attributed to failure of pairing, partial or complete, in the distal segments of the chromosomes and this failure again to differential precocity (see below) preventing the pairing of parts of chromosomes which have divided before the rest (Darlington, '31b). Whatever its mechanism, localization, in regard to which species of *Fritillaria* differ, is a genetic property. Such a property has been found to control the distribution of crossing-over in *Drosophila* (Bridges, '29; *cf.* Darlington, '31a). It may be noted that the differences in distribution between the different chromosomes in *Drosophila* must clearly be structurally determined. They are perhaps related differences in the spacing of the hereditary material in its supporting framework—differences of which the visible signs are the constrictions.

Another property affecting chiasma distribution is interference (Haldane, '31b). It is probable that races differ genetically in this respect (Darlington and Janaki-Ammal, '32).

(viii) *Terminalization of Chiasmata.* Related species differ in the degree of terminalization of chiasmata (*e.g.,* in *Tradescantia,* Darlington, '29b), although as a rule large groups are fairly constant in this property: the Hemiptera, Lepidoptera, Tettigidae and the Onagraceae and Solanaceae, generally have a high degree of terminalization; the Acrididae and the Liliaceae, the Gymnosperms and many Leguminosae and Rosaceae have little terminalization. There is no evidence as yet of great differences within the species (but *cf. Fritillaria,* Darlington, '30). Nevertheless, amongst many conditions of terminalization that are now coming to light none have been found to account for the radical differences found between different pure species on any other than

a genetic basis. It must therefore be supposed that species differ in genetic conditions determining the degree of terminalization.

(ix) *Precocious Condensation of Chromosomes.* Although the property of differential condensation is almost entirely confined to the stage of meiosis and to chromosomes (actually sex chromosomes) which have the exceptional property of not pairing at this stage, there is evidence to show that the lack of pairing does not usually determine this behavior, but that a genetic-environmental reaction may do so.

Thus chromosomes may show differential condensation at pre-meiotic divisions (Brunelli, '10, '11; Boveri, '11 *et al.*). An unpaired X-chromosome which shows condensation in the testis does not show it in exceptional ovarian tissue in the male of *Perla marginata* (Junker, '23). Such differential behavior probably determines failure of pairing in the spermatogonia of *Rhabditis* (Boveri, *l.c.*). In these cases an environmental (developmental) condition determines the difference in behavior of particular chromosomes; thus the possibility of its being due to a structural difference or a lack of partner is excluded. If this may therefore be a genetic property we have an alternative explanation for its general correlation with failure of pairing. The condensation may be the cause, not the effect; for failure of pairing between chromosomes carrying the sex-factors, if determined in this way, would separate the corresponding chromosomes phylogenetically and thus permit of the origin of differences which would later in themselves prevent pairing (*v. infra*).

This differential property of certain chromosomes like that referred to above, of parts of chromosomes, is significant from another point of view. It is difficult to suppose that such differential behavior is a reaction of the specific hereditary material which is specialized for an entirely different function. The reaction therefore points to the existence of associated materials of non-

genetic function (such as are also responsible for the constrictions and other discontinuities referred to above). It therefore provides evidence for a third factor in chromosome behavior, *viz.*, properties of *accessory* materials, other than the essential hereditary materials, or unessential conditions of these materials in the chromosome.

(x) *Polarization of the Nucleus at Zygotene.* Little is known of the distribution of this property, owing to difficulties of observation at the early prophase of meiosis. Consequently no differences in regard to it are recognized between closely related forms. Of the two best known groups one, the Orthoptera, has regular polarization, the other, the Liliaceae, has none.

(xi) *Occurrence of a Diffuse Stage in the Prophase of Meiosis.* This property is widely distributed in animals but is never found in plants. It does not seem to affect the eventual behavior of the paired chromosomes and is not therefore of interest to us at present.

(xii) *Suppression of Mitosis, Nuclear Separation, Division of Chromosomes, etc.* Factors affecting the mitotic mechanism are well recognized and do not affect the present problem closely. Of genetic significance are those which must be supposed to determine:

(a) Syndiploidy in lizards and grasshoppers (Painter, '21; Eisentraut, '26) in *Brassica japonica* (Fukushima, '31) and in *Zea Mays* (Beadle, '30) where it is associated with genetic failure of meiosis.

(b) Fusion of nuclei after the second division or in an early segmentation division in many cases of diploid parthenogenesis with normal meiosis (*e.g., Rhabditis,* Bĕlař, '23). This property can arise as a mutation (P. Hertwig, '20).

(c) Supernumerary mitoses in the pollen grain of *Zea Mays* (Beadle, '31). This is proved to be a recessive character.

(d) Failure to form daughter nuclei regularly after meiosis in strains of *Triticum vulgare* (L. A. Sapehin,

'31) and in species of *Kniphofia* (Moffett, unpublished), a condition probably related to male sterility, which is determined by a mendelian factor in many species.

(e) Polarization of the embryo-sac in the *Caninae* roses, by virtue of which all unpaired chromosomes usually pass to one daughter nucleus (Täckholm, '22).

(f) The reduction of the X-chromosomes at the abortive meiosis in male-producing parthenogenetic aphides (Morgan, '15).

The supernumerary mitoses in *Zea Mays* seem to be of theoretical importance. The prophase of the first of these divisions begins before the chromosomes have divided, and at metaphase the chromosomes show an excessive linear contraction. In both these respects the division resembles meiosis. It can only resemble the meiosis of a haploid, and the occasional pairing at metaphase, by interstitial chiasmata, is therefore particularly significant. This is probably analogous to the pairing observed in haploid *Oenothera* by Emerson ('29). It may be supposed to result in both cases from the pairing of reduplicated segments in the hybrid set, which do not normally get a chance to pair on account of not being in the same linear sequence. The structure of the interstitial chiasmata suggests that the chromosomes have divided during the prophase, as at meiosis, and in the four or five divisions which rapidly succeed one another their halves must be supposed to separate in order to give as many as 32 cells. The genetic condition therefore seems to resemble that determining meiosis, with two differences: (1) It acts on haploid nuclei and therefore can not give regular reduction, (ii) it stimulates not two but a whole series of divisions.

The effects of environmental (and developmental) differences have already been considered in so far as they merely condition the expression of genetic differences. Other environmental differences have effects on the chromosomes as on other organs parallel to those of genetic differences—presumably because they are physio-

logically analogous. This parallelism is important be-
cause where it occurs it excludes the possibility of a
structural basis for the variations concerned. Thus
changes in temperature and application of various re-
agents will (i) increase the degree of prophase contrac-
tion of chromosomes (Delaunay, '30), (ii) suppress or
modify the anaphase separation of chromosomes at
mitosis and meiosis (Sakamura and Stow, '26; Nemec,
'29, *et al.*). Other changes in external or developmental
conditions will (i) change the amount of crossing-over
(Plough, '17; Bridges, '29) (ii) change the frequency of
chiasmata in a chromosome pair (*cf.* Darlington, '31b,
Fig. 6) (iii) change the frequency with which chromo-
somes pair in a hybrid (Kihara, '29) and thus sometimes
abolish pairing altogether locally (Meurman, '29, on
Ribes).

Environmental changes have not yet been shown to
influence (i) the bulk of chromosomes individually or as
a complement at metaphase, (ii) the position of the
attachment constriction, (iii) the formation of multiple
chromosomes or their disintegration, (iv) the localization
and terminalization of chiasmata. Their effects cover
an especially narrow range as compared with those of
genetic differences, presumably because they can operate
less directly and less specifically on the chromosomes
than on the gross structure of the organism. They
merely provide one measure of the degree of elasticity
of the mechanism. Environmental effects are significant
in relation to evolutionary theory in one respect: unlike
effects on the gross structure they are never of an adap-
tive character. The adaptations in chromosome be-
havior to be considered later are therefore especially
cogent evidence against the Lamarckian hypothesis.

Note. The failure of the division of chromosomes,
leading to their elimination and the formation of mosaics
in *Drosophila* and Maize (Bridges, Stern, Stadler, *et al.*),
are more probably due to structural peculiarities of the
chromosomes concerned. Since the supernumerary chro-

mosomes of *Camnula* seem to show a special propensity
for irregular distribution (Carroll, '20) their origin is
similarly to be attributed to irregular division of one of
the normal chromosome complement associated with a
structural peculiarity.

These observations enable us to classify the sources of
variation in chromosome behavior under four heads: (i)
Changes in the permanent (hereditary) structure, (ii)
changes in the genotype, (iii) changes in the "accessory
materials" in the chromosomes, (iv) changes in the
environment. The first and fourth controlling factors
have long been recognized; the third can not yet be
exactly estimated or even defined; but the second is a
factor whose importance can now be considered.

II. Deductive

The importance of the inference of genetic control is
twofold.

(i) *A distinction between genetic and structural causes
of differences in chromosome form and behavior is neces-
sary for deciding their evolutionary significance.*

The observations in question are of two kinds:

(a) Comparative observations of chromosomes at
mitosis in related species and varieties: from these the
relationship of the species is deduced. Clearly it is im-
portant to know whether a difference in the size of
chromosomes is the result of the action of a single genetic
factor or of the accumulation of structural changes each
of which would have an important genetic effect. In the
present state of our knowledge conclusions must be ten-
tative, but it is easiest to suppose that the differences in
chromosome size between *Nicotiana* and *Solanum*, be-
tween *Tradescantia virginiana, T. crassifolia* and *Cyano-
tis,* and between different sections of *Lactuca, Esch-
scholtzia* and *Campanula* are determined largely by
genetic factors, while the difference between *Nicotiana
alata* and *N. sylvestris,* between *Tradescantia virginiana*
and *T. navicularis* and between different species of
Gryllus (Ohmachi, '29) are determined by structural

changes—fragmentation or fusion (refer to Tischler, '31).

It is of course difficult to estimate the relative importance of structural and genotypic change in determining variation in the bulk of chromosomes in living organisms as a whole; but their relative range of effect may be tentatively estimated. Differences within a complement are wholly due to structural differentiation, since the same genotype controls the whole complement. The highest range found in a complement is in the relation of about 1 : 50 (*Muscari latifolium*, Delaunay, '29). The highest range between the *largest* chromosomes of two complements is found within a single order of Dicotyledons, the Droseraceae, and is between volumes in the proportion of 1 : 1000. This difference must be almost wholly genotypically controlled.

The two ranges are both effective ranges, and neither of them represents the theoretical maximum, which is necessarily much greater for structural change. But the comparison shows that in effective range genotypic control is probably the more important source of variation.

Note. The chromosomes of *Aesculus Hippocastanum* are one eighth the size of those of *Ae. pavia* and its section of the genus. In the presumed hybrid *Ae. carnea* the distinction is retained (Skovsted, '29). It can not therefore be determined by the genotype. Yet it seems impossible to suppose that so uniform a difference has arisen through structural change. Here then seems to be a second kind of evidence for accessory substances in the chromosomes as a third factor in their behavior.

(b) Observation of *meiosis* in hybrids: from these, on the assumption that the frequency of pairing is a measure of affinity, extensive studies of species-relationships have been carried out in *Triticum, Nicotiana* and other genera. But the pairing is only an indirect measure of the frequency of chiasma formation (Darlington, '31b), and this in turn is an unknown measure of the length of chromosome paired at pachytene and of the effect of genetic

factors. This length is conditioned by identity of the
materials making up the chromosome and the identity of
their arrangement—as shown by Dobzhansky's observa-
tions ('31) on the reduction of crossing-over in transloca-
tion heterozygotes (*cf.* Darlington, '31c). A frequent
lack of relationship between the "affinity" of species and
of their chromosomes is therefore to be expected, and is
very often found (*cf.* Federley, '14).

It will be seen therefore that these observations of
affinity, while of great theoretical interest, can not be
applied to the special problem of phylogeny without a
consideration at least of conditions of chiasma formation
in the parents as well as in their hybrids. While the
forms of bivalents (indicating the frequencies and distri-
bution of their chiasmata) have been illustrated in *Triti-
cum* and *Aegilops* hybrids with remarkable accuracy
(Kihara, '29, *et al.*) there is no illustration whatever to
show comparable conditions in their parents. A method
of approach to this problem has been described elsewhere
(Darlington, '31d).

(ii) *The assumption that chromosome form and be-
havior are subordinate to the genotype is the remaining
postulate necessary for the establishment of a single
inductive-deductive system in which all observations of
chromosome form and behavior can be consistently
arranged.*

With the assumption that chromosome form and be-
havior is subject to genotypic control its variation
amongst species can be considered as adaptive. Adapta-
tion must be assumed to be brought about by natural
selection of variations on the Darwinian principle. (The
Lamarckian hypothesis is excluded by the considerations
given above, as well as on general grounds). The re-
sults of study from this point of view are indispensable
to evolutionary theory, since the variations observed in
some measure control such properties as sexual reproduc-
tion, sexual differentiation, parthenogenesis and poly-
ploidy. The following may serve as examples.

(i) Small chromosome size is an adaptation to polyploidy, which has only reached a high development in the dicotyledons with small chromosomes. It is also necessarily a concomitant of small cell-size. The hereditary material can not be supposed to be essentially different in organization in different groups of insects and flowering plants, in accordance with the great size differences observed. The chromosomes of the Liliaceae and Orthoptera would be mechanically incompatible with the cells of the Rosaceae and Lepidoptera. Genetic variation may therefore be supposed to have led to adaptation of chromosome size to cell size.

Since cell size and chromosome size are independent in the sources of their variation, but associated in its expression, it is not possible to determine at once which is the limiting factor. But variation affecting cell-size is of universal occurrence. It has undoubtedly played a part in the origin of most polyploid species, since these have not the cell-size proportions that are found in new polyploid forms. Genotypic variation in chromosome-size, on the other hand, seems to be absent in many large groups (such as the Rosaceae and the Lepidoptera). Where it occurs it is usually found to lead to a wide range of sizes such as the three distinguishable in the Droseraceae and the Tradescantiae. Probably therefore we may look to genotypic variation in chromosome size as a controlling factor in cell-size variation.

(ii) Aggregation of small chromosomes is an adaptation which secures regular pairing without high chiasma frequency. High chiasma frequency may have two disadvantages, (a) on the chiasmatype theory it means high crossing-over (which will tend to reduce heterozygosis in a population when lethal or depressive mutations occur), (b) in the absence of terminalization (before metaphase) it leads to lagging of the paired chromosomes at anaphase with possible irregular disjunction where the bivalents differ. It is therefore interesting to notice that

this aggregation is only retained in *Ascaris* in those chromosomes which are to undergo meiosis. This point of view leads us to consider that fragmentation and diminution do not themselves determine the germ track but are parallel genetic-developmental effects to that determination.

(iii) High chiasma frequency (with high crossing-over on the chiasmatype hypothesis) secures the most rapid distribution and recombination of variations in a species. But unless combined with some degree of terminalization it leads to the most difficult separation of chromosomes at anaphase. It is to this that the wide-spread, probably universal, occurrence of some degree of terminalization is to be attributed, and especially its characteristic occurrence in natural ring-forming genera such as *Oenothera, Rhoeo, Anthoxanthum* and *Campanula* (cf. Darlington, '31b).

(iv) Polarization of the chromosomes at zygotene will make for regularity of pairing in any organism and will considerably modify the results of pairing in hybrids. Any change in the linear homology of the chromosomes will interrupt their pairing at zygotene, as may be inferred not only from direct observation but from crossing-over frequencies (Dobzhansky, '31). If pairing therefore begins at the ends, the segments whose pairing will suffer will be the proximal ones. This is particularly important in ring-forming complex-heterozygotes whose complex differences are probably localized near the middles of the chromosomes (Darlington, '31c, App. V.). It is probable therefore that *Oenothera, Rhoeo* and other genera of this kind have a polarized nucleus with a "bouquet" stage—although this has not yet been shown. It may be mentioned, by the way, that polarization limits the exchange of partners at zygotene and so probably prevents the formation of multivalents where they would otherwise be expected—as in the polysomic spermatocytes of *Camnula* (Carroll, '20; cf. Darlington, '31d).

(v) Crossing-over is generally restricted (in sex chromosomes and autosomes) in the heterozygous sex in animals (Haldane, '22). Since genetic factors affecting chiasma frequency and distribution seem to affect all the chromosomes alike, it seems probable that factors restricting and localizing crossing-over have been selected in the heterozygous sex. The same process must be the basis of secondary sexual differentiation, for only by the abolition of crossing-over between the original homologous sex factors and others in the sex chromosomes establishing secondary differences can these differences become permanent (Darlington, '31a). Possibly in some species the same end has been attained by the selection of factors which hasten the prophase development of the sex chromosomes differentially and thus inhibit their pairing.

(vi) Most sexually fertile species show a remarkable uniformity in the size of the chromosomes making up their complements. This can not be accounted for by the known randomness in the occurrence of changes in their structure. Rather, since the pairing of chromosomes seems to be determined by the formation of chiasmata, this uniformity is necessary for their regular pairing if the chiasmata are regularly distributed in proportion to the length of the chromosomes. When therefore great differences are found in the size of the chromosomes making up a set, this is associated with a greater frequency of chiasmata in proportion to their length in the short chromosomes (*e.g.*, in *Hyacinthus amethystinus,* unpublished). Alternatively, where this is not the case, the small chromosomes fail to pair and do not form a regular part of the complement (*e.g.*, fragments in *Tradescantia* and *Fritillaria, cf.* Darlington, '31b) or they pair momentarily at metaphase by an exceptional method, probably found only in the Hemiptera. Wide variation in chromosome size in the permanent complement is therefore conditioned by genetic properties of the organism in regard to chiasma-formation. In this regard it is interesting to

notice that in *Gryllus,* where the autosomes have undergone fragmentation, the X-chromosomes are unaffected, presumably because chiasmata are formed less frequently in them (Ohmachi, '29; *cf.* also *Orphania denticauda,* de Sinéty, '01).

(vii) *Causation of meiosis.* Meiosis must be supposed to have arisen as an aberration of mitosis. Since this must have occurred in the rather remote past it may seem absurd to speculate as to its conditions. But three considerations make it reasonable: (i) Both meiosis and mitosis preserve certain characteristics which being universal must be supposed to have been originally present. (ii) Meiosis must have arisen in one step from mitosis, for unless at the first attempt it gave an accurate reduction it would only destroy the cell-generation that led up to it.   (iii) Genetic conditions have been revealed in two genera which as far as possible reconstruct the scene.

In *Matthiola,* meiosis appears to start so late that the chromosomes do not contract as much as normally, and pairing is liable to failure.   In polymitotic *Zea,* mitosis appears to start too early, so that the chromosomes contract more than normally and some pairing occurs.   The abnormalities both in *Matthiola* and in polymitotic *Zea* therefore indicate that meiosis is determined by a premature prophase interrupting the resting stage before the chromosomes have divided and leading to their pairing, chiasma-formation (crossing-over) and a greater contraction of the chromosomes at metaphase (*cf.* Darlington, '31b).   The occurrence of meiosis at a particular stage of development we can therefore regard as determined by genetic factors and as an adaptation necessary for sexual reproduction.   The evidence for this *precocity* hypothesis has been systematically examined in the contribution referred to (1931b).

(viii) The distinction between structural and genotypic effects enables us to analyze the conditions of the reverse change, by which parthenogenesis and other forms of apomixis have replaced sexual reproduction,

more exactly than has been possible hitherto. These seem to be the following:

(a) The genetic property of permitting *either* the development of the specialized female germ cell without fertilization (haploid or diploid parthenogenesis) *or* the development of unspecialized tissue into an embryo (apospory, apogamy, nucellar embryony).

(b) The property of producing, in the first type, unreduced germ cells and, in the second type, inviable germ cells. This may be either (i) genetic (as pointed out above) (ii) structural, *i.e.*, depending on the structural relationship between chromosomes of opposite parents in a hybrid (especially in the second type), or (iii) a combination of the two. The second condition probably always leads to the third and is responsible for the complexity of many cases in plants. Thus it is possible for a sexually sterile interspecific hybrid having the necessary genetic conditions to be, parthenogenetically, fairly fertile, owing to the formation of unreduced germ cells in the way shown by Rosenberg ('27). Unreduced gametes will, in the original hybrid, be formed rather irregularly, as found in *Ochna serrulata* by Chiarugi and Francini ('30). Their formation is associated as might be expected, on the precocity theory of meiosis, with a time irregularity: it anticipates normal meiosis by five days. But gametes formed in this way can show segregation on a lower scale than normal mendelian segregation, owing to the occasional pairing of chromosomes, crossing over between them and consequent segregation at the division of restitution nucleus. This will be important because the rarer the pairing the greater, we must suppose, the differences to be segregated. Segregation should therefore take place in a new parthenogenetic hybrid on a scale appropriately reduced. If the occurrence of meiosis is affected (as we know it may be) by genetic factors in regard to which segregation takes place, then secondary adaptation will occur leading to the total and regular suppression of meiosis, since segregates

having such suppression will be more fertile. Mutation found by Ostenfeld ('20 *et al.*) in parthenogenetic *Hieracia* is a proof of the occurrence of the processes I am imagining and, whether or not it has the cause I suggest, its result will be the same.

III. CONCLUSION

The assumption of continuity and permanence in the structure of the chromosomes, combined with a potentiality for random change, allows the consideration of chromosome form and behavior from the point of view of adaptation and evolutionary theory to only a limited extent. This is due to the fact that changes in chromosome structure and number must as a rule be vastly more important in their genetic effect on the organism than as adaptations to their mechanical function. Two notable exceptions suggest themselves. Polyploidy and ring-formation provide the means by which hybrids (of sudden and of gradual origin, respectively) may become true-breeding species (Darlington, '28, '29a). With these exceptions we may say that where structural changes are concerned, adaptation of the chromosomes to their mechanical functions is subordinate to adaptation to their physiological functions.

But the assumption of genetical control allows the theory of adaptation to be carried to its logical conclusion. Some of the examples of adaptation cited above have long been recognized and in the absence of the principle of genetic control they have been the subject of a variety of explanations. These are analogous to the explanations attempted in regard to evolution in general as alternatives to the adaptive principle which has been applied here. They may be classified (neglecting fallacies, such as telosynapsis, which are based on erroneous observation) as follows:

(i) *By teleology.* Reduction of chromosome number at meiosis and therefore the occurrence of meiosis was customarily explained by the older investigators as having the *purpose* of compensating for the addition in fer-

tilization. Similarly, it has been suggested that polyploids owe their origin to chromosomes doubling their number in order that they might have partners.

(ii) *By false analogy.* The differential condensation of the chromosomes has been regarded as a symptom of degeneration and the sex chromosomes have been sup, posed to undergo such changes on the analogy of the evolutionary processes which are inferred in imperme-a-nent structures. Similarly, processes of "historiation·m," "quantitation" and the like have been assumed ɔu in chromosomes (Delaunay, '30; Navashin, '31, *et al.*). Such descriptions skip a necessary step in the argument/ and imply inherent changeability. Yet the chromosomes do not change of themselves but rather through the accidents of their relationship with external mechanical forces (as the primary changes in irradiation) and with one another (as in the secondary changes in crossing-over). Such terms therefore contradict the theory of continuity without evidence, and leave us in an inconsistent position.

(iii) *By petitio principii.* Reuter ('30) has avoided both these fallacious short cuts and attempted to arrive at a purely causal explanation of chromosome behavior. This consists in the assumption of "rein bio-physikalisch-chemische Kräftespiele," which determine chromosome behavior. Then, if the proper forces are assumed (such as agglutination, affinity and the like) the observed results ensue. This method of argument makes the earlier fallacies unnecessary and brings us back to where we started. The examination of chromosome behavior by this method seems to have been entirely fruitless. The Kräftespiele remain an enigma.

Begging the question in this way is not the beginning of an explanation. We must not attempt to consider ultimate "bio-physico-chemical reactions" until we have determined immediate conditions. This is possible by genetical analysis, and the present attempt, faulty as it no doubt is, shows the only direction along which inquiries of causal relationships can be profitably pursued.

SUMMARY

It is shown that in twelve different ways the behavior of the chromosomes of an organism in the resting nucleus, in mitosis and meiosis, is subject to the control of the genotype. The chromosomes are therefore subject as a whole to the variation in their parts. This qualification of the principle of continuity, first, makes it necessary to consider all variation in chromosome behavior as the result of either genotypic or structural change and, secondly, makes it possible to examine this variation in terms of evolutionary theory, considering it in every detail as the product of an adaptive process. Such a point of view is important in considering the origin of meiosis, sexual dimorphism and parthenogenesis. Some of the genotypic differences observed raise questions of physiological interest (affecting, for example, the organization of the hereditary material in the chromosome) but these are not yet ripe for discussion (*cf.* Haldane, '32). The problem will be dealt with more fully elsewhere.

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THE NINE PRINCIPLES OF EVOLUTION REVEALED BY PALEONTOLOGY[1]

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IN honor of Darwin our first thought is that Natural Selection is the sole survivor of the age-long theories and hypotheses clustering about evolution. When we consider the youthful zoology and the infantile paleontology of Darwin's time (1809–82), our admiration for his genius and marvelous powers of generalization constantly increases. What would his generalizations have risen to with our present knowledge? The ratio of the 8,767 vertebrate species known in his time to the 65,939 species known in 1925, nearly 8 to 1, is about the measure of the biological progress of the first century of evolution. In 1831 only three species of fossil elephants were known—the Mammoth (*Elephas primigenius*), the Mastodon (*Mastodon americanus*) and the southern mammoth (*Elephas meridionalis*); now there are over 350 species and over 30 genera. Darwin foresaw the promised land of paleontology, but did not live to enter it.

It is a striking fact that the zoologists, experimentalists and geneticists who, a quarter century ago, were stoutly combatting Wallace, Weismann, and other superselectionists, have, one after another, returned to the Darwinian sheepfold and are now almost unanimously teaching their students, as if it were a demonstrated fact, that evolution progresses by the survival of fortuitously adaptive mutations. To the mind of the paleontologist these teachings are pure Darwinism camouflaged in new language. Bateson, founder of the genetic school, is the only one to confess frankly his utter failure to explain the origin of species; few have displayed similar courage.

[1] Fourth paper in the "Centenary of Evolution" Conference before the British Association, September 24, 1931. This is the sixth of a series of papers by the author on the origin of species; the seventh will be entitled "The New Concept of Evolution."

Selection alone has stood the test of survival of the fittest, yet we must severely limit the powers of selection as Darwin imagined them in his earlier and more sanguine frame of mind, and glean the elements of truth pervading all the other hypotheses and theories.

GENETICS A SCIENCE OF HEREDITY, NOT OF EVOLUTION

The geneticists are trying to make evolution fit the genes rather than to make the genes fit evolution.

Far from marking any real progress toward the eternal question of the origin of coordinated mechanical adaptations, the experimental zoologist and geneticist are making little advance along the biochemical or experimental lines. In order to ascertain the state of current observation and opinion on the mutational origin of species, sub-species or adaptive mutations, as well as on the experimental results of biochemical, biophysical and endocrine action, the following questionnaire (slightly modified) was sent out on July 17, 1931, to forty-one leading zoologists:

1. Do you know of a single concrete case of the origin of a species, of a sub-species, of an adaptive mutation of De Vries, or of a single adaptive character, arising suddenly by mutation or saltation either under natural or experimental conditions?

2. Granted that permanent hereditary sports, mutations and modifications of existing characters are being produced by biochemical and biophysical means, has a single adaptive character been produced by such means through experiment in your laboratories?

3. Granted that profound changes in coloring, in form, in somatic proportion, in the developmental acceleration or retardation of characters, may be produced by glandular action, is there sufficient evidence that Nature has ever proceeded in this way except in producing immunity and non-immunity?

Up to August 13 seventeen replies had been received. To Questions 1 and 2 the prevailing answers were negative as indicated by the reply "No." More or less positive or affirmative replies were also received to Question 1, but analysis of most of these replies indicates that the crucial element in Question 1 is evaded, namely, *the ori-*

gin of adaptive characters. No doubt certain mutations
do survive; many of them are recorded in the answers to
the questionnaire; Crampton has discovered mutations
in his monographic researches on the mollusc *Partula;*
Chapman in the avian genus *Buarremon;* the short-
legged Ancon sheep is a classic; certain biomechanical
mutations in the pelagic Ciliata, such as the spiral shelf
of *Xystonella scandens* observed by Kofoid, may be of
sudden or mutational origin, although this is not proved.
The answers of seventeen zoologists to Question 2,
as to adaptive biochemical origins by experiment, were
uniformly negative; new and hereditarily permanent
mutations may be aroused by more or less violent chem-
ical or physical means, but not a single one is known
to be adaptive. The answers to Question 3 were five
negative and five affirmative; the negative squarely meet-
ing the question, the positive reaffirming the granted
postulate that endocrine secretions profoundly modify
all existing characters and processes of development, but
not a single case can be cited wherein a new biomechani-
cal character has arisen through endocrine action.

Zoology, Comparative Anatomy and Embryology Reveal Ten Principles of Evolution

Although primarily an original observer rather than
a collator of other people's ideas, Darwin was more or
less familiar with ten of the principles of ''biomechani-
cal'' adaptation which had been observed in the hard
parts of animals from the time of the earliest Greek
anatomists and philosophers. Throughout his frequent
discussions of the biomechanical evolution of animals
are included the bony and muscular biomechanical adap-
tations brought about through processes of (1) degen-
eration, (2) development, (3) compensation, (4) econ-
omy, (5) change of proportion, (6) coadaptation, (7
and 8) acceleration or retardation, (9) self-adaptation,
and (10) sports and discontinuities.

Note that all these ten pre-Darwin principles of adaptation relate not to the *origins of organs,* but to the *modification of existing organs,* as of the wings of the duck, the neck of the giraffe, the speed of the wolf. Darwin realized that the weak point in his theory was in the matter of origins; he could, and did, largely explain the survival value of organs once established, but was hard put to place a survival value on fortuitous variations.

These ten zoological principles are as follows:

ZOOLOGIC MODIFICATION OF EXISTING ORGANS, ONTOGENY

Discovered in Anatomy and Embryology.

Known to Darwin.

1. Biomechanical *onto-retrogression* (Aristotle), degeneration, atrophy of organs.
2. " " *-progression* (Aristotle), development, hypertrophy of organs.
3. " " *-compensation* (Aristotle), metatrophy and eutrophy of organs.
4. " " *-economy* (Aristotle), metatrophy and eutrophy of organs.
5. " " *-allometry* (Lamarck-Darwin), allometrons, changes of proportion in organs.
6. " *co-adaptation* (St. Hilaire), coordination, correlation of organs.
7. " *onto-acceleration* (v. Baer) into earlier growth stages of organs.
8. " " *-retardation* (v. Baer) into later growth stages of organs.
9. " *auto-adaptation* (Goethe) through principles 1-8.
10. " *onto-saltation* (St. Hilaire), sports, discontinuities.
11. *Cellular continuity of the germ plasm-in the perpetuation of organs* (Weismann).

One great principle remained to be established after Darwin's time, namely, (11) Weismann's cellular continuity of the germ plasm, in antithesis to all pangenetic or somatic hypotheses of the origin of hereditary characters. To Weismann's principle of cellular continuity paleontology adds the following nine new principles of germinal evolution.

PALEONTOLOGY REVEALS NINE NEW PRINCIPLES OF GERMINAL EVOLUTION

The "biochemical" adaptations underlying coloration, as well as the "biophysical" adaptations controlling many of the animal instincts, are beyond the ken of the

paleontologist who is bounded by his biomechanical fossils. Nevertheless, paleontology is the acid test; *paleontology is evolution.*

In this struggle for existence of bygone theories and of new hypotheses, it seems that paleontology, with its world of new and wholly undreamt-of evidence as to the *origin of adaptive biomechanical characters,* serves as the two-edged sword of biology; it cuts hypotheses unfit to survive; it strengthens hypotheses fit to survive. It calls for conceptions of a new and synthetic physicochemical order to supplant outworn hypotheses dating back to Empedocles (600 B. C.). Paleontology disestablishes the entelechy hypothesis of Aristotle (300 B. C.) and of all his "vitalistic" followers like Driesch and Bergson. It substitutes for Aristotle's "internal perfecting tendency" the idea of adaptive reaction and interaction of internal with external energies which has been formulated (Osborn, 1912–1929) into a new *tetraplastic* principle of the "four inseparable energetic factors of evolution," namely: (1) physical environment, (2) ontogeny including habit, (3) living environment, the biota, (4) the germ plasm or *geneplasm.* The above energetic complex is subject to the non-energetic selection— survival of the fittest.

This tetraplastic principle which seeks to combine the elements of truth in preceding hypotheses and theories has, thus far, won no acceptance.

In causation of the origin of species and subspecies, paleontology unites with modern field zoology in firmly establishing the direct action of environment (Buffon, 1755–Wagner, 1870) on the germ plasm as a causative factor. It disestablishes the habit-inheritance law of Erasmus Darwin and of Lamarck (1790–1809); through auto-adaptation it establishes habit as a *guiding* principle in evolution, but not in the Lamarckian sense. Paleontology eliminates selection from the energetic complex; it establishes the non-energetic selection as a universal and outstanding guide and principle of progress

from the beginning of time; it disestablishes the third and fourth principles of Charles Darwin (1859), namely, of the origin of adaptations through the survival of the fortuitously adaptive. It firmly establishes the inconspicuous adaptive origins of new characters first observed by Waagen (1869), which may be distinguished as *W.* mutations when compared with the fortuitous *D.* mutations of De Vries (1911). At least from biomechanical evolution it excludes entirely the fortuitous *D.* mutations of De Vries. It helps to disestablish the "pangenesis" of Darwin and all similar theories of the somatic origin of adaptive characters; it accordingly disestablishes the "inheritance of acquired characters" and establishes the complementary principle of the "continuity of germ plasm" of Weismann (1880). It disestablishes the superselection theories of Weismann and other neo-Darwinians. In its earliest (1806), as well as its latest phases (1931), paleontology undermines the primitive idea of "created evolution"; of recent years it tends to establish the wholly different idea of "creative evolution," which may be provisionally termed *aristogenesis*. Finally, paleontology unites with systematic and experimental zoology in compelling us to concentrate research on the origin and coordination of biomechanical characters, in the lower animals and in man, as the outstanding problem of the second century of evolution.

Paleontology (1869–1931) through a succession of discoveries has revealed nine principles of *adaptive biomechanical origin in the germ plasm.* Thus, up to the present time, we have established through combined observation in zoology and paleontology no less than twenty more or less distinct but invariably cooperative principles of biomechanical adaptation.

Of the nine principles of biomechanical origin discovered in phylogeny since Darwin's "Origin of Species" (1859), the first three were observed in fossil invertebrates, namely: (12) the *D.* mutations of Waagen (1869), in which is involved (13) the "trend" or "mutations-

PALEONTOLOGIC ORIGIN OF NEW CHARACTERS, PHYLOGENY

Discovered in Paleontology.		Not known to Darwin.		
	12.		Biomechanical *phylo-mutation* (Waagen, 1869), orthogenesis in new characters.
	13.		"	*-trend* "mutations-richtung" (Neumayr) in new characters.
	14.		"	*-acceleration* (Hyatt, 1880) in the evolution of single characters.
	15.		"	*-retardation* (Hyatt, 1880) in the evolution of single characters.
	16.		"	*-continuity* (Osborn, 1889–1931) vs. discontinuity in single characters.
	17.		"	*-potentiality* (Osborn, 1889–1931) in the origin of new characters.
	18.		"	*-predetermination* (Osborn, 1889–1931) in the origin of new characters.
	19.		"	*-rectigradation* (Osborn, 1889–1931) in the origin of single characters.
	20.		"	*-aristogenesis* (Osborn, 1889–1931) in the rise of grouped characters.

richtung" of Neumayr (1875); (14–15) the "acceleration and retardation" of Hyatt (1880), principles which Darwin could not clearly comprehend. In the year 1889 Osborn, at the time a convinced neo-Lamarckian, began his extremely intensive observations upon the origin and development of single adaptive characters, aided by unprecedented fossil material first in the primates and then in five independent divisions of the ungulates, wherein were revealed five previously undiscovered principles of biomechanical adaptation, namely: (16) germinal continuity versus discontinuity, and W. mutations ascending and descending; (17) germinal potentiality, as a basis of Lankester's principle of *homogeny;* (18) germinal predetermination, as distinguished from indeterminate origins; (19) germinal rectigradations, as distinguished from fortuitous or chance origins. Finally, in the synthesis of the orthogenetic origin of new adaptive characters in all the mammals, including man, there was reached (20) the grouped principle now provisionally termed *aristogenesis* for the want of a more appropriate word to express continuously creative adaptation.

A very important distinction is observed between *rectigradations* which are predetermined, and changes in proportion, as in the elongation of the neck of the giraffe, technically known as *allometrons,* which are not prede-

termined. Rectigradations are relatively rare, while allometrons, or changes of proportion in the teeth, skull and limbs, are constantly in progress and make up a larger part of the definition of species. Similar rectigradations arise through community of descent. Similar allometrons are constantly arising in animals of dissimilar ascent.

Whereas the zoologist, comparative anatomist and geneticist, by the very nature of the evidence at their command, find difficulty in distinguishing between the accidental, fortuitous and temporary variations, fluctuations and *D.* mutations, the paleontologist is absolutely sure of his footing as soon as he is enabled to observe the ascending geological *W.* mutations of animal mechanisms, whether invertebrate or vertebrate. He advances solely by inductive means, after the manner of Darwin. If he is not sure of the adaptive trend of a certain rectigradation in its feeble, incipient stage, he may observe it a hundred thousand or a million years later as the dominant and commanding character of the entire organism.

As Weismann spoke of the immortality of the germ plasm, the paleontologist may speak of the secular immortality of thousands of characters which he is enabled to observe through the whole cycle from potentiality and predetermination in the germ plasm until, after eons of use and service, they may subside again into the mysterious germinal substance—mysterious because utterly inexplicable. The biomechanism of the titanothere and of the elephant is due to a complex of energetic factors which is entirely beyond our present comprehension.

Conclusions

The paleontologist concludes that *the origin of species,* so far as species are defined by various stages of biomechanical adaptation, has long ceased to be a problem; the manner by which subspecies, species, genera, families and orders arise through divergence, acceleration, retardation and rectigradation is also perfectly clear.

One after another the original Buffonian, Lamarckian, Darwinian, Weismannian and De Vriesian theories of causation have failed.   Each, however, contains elements of truth.   As to Lamarckism, we can affirm that it is the essential living principle of biochemical reaction which as a secular effect calls forth the adaptive biomechanical response, whether in ontogeny or in phylogeny.   As to Darwinism, selection acts incessantly; it originates nothing, it does not control the origin of characters; it may or may not control the rate of evolution.

All that we can say at present is that Nature does not waste time or effort with chance or fortuity or experiment, but that she proceeds directly and creatively to her marvelous adaptive ends of biomechanism.

Darwin was at once naturalist, geologist, paleontologist, zoologist, botanist.   To this galactic universe of talents, he who would generalize from the twenty principles of biomechanical adaptation must also be chemist, physicist and philosopher.   We await the man of genius to discover the *causes of evolution* according to an entirely new concept of evolution.

While we know infinitely more about the modes of evolution than did Charles Darwin, and while we can demonstrate beyond refutation the prevailing twenty principles of biomechanical adaptation discovered in ontogeny and phylogeny, *we are more at a loss than ever before to understand the causes of evolution.*

ON THE ORIGIN OF MUTATIONS

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I

A REVIEW of the modern work in genetics shows that many mutations both in animals and in plants have been discovered. How do these mutations arise? This is the great question in genetics.

What may prove to be a great step forward in advancing our knowledge of the mechanism of Mendelian heredity was Muller's discovery that Roentgen rays can induce mutations in *Drosophila*. Subsequently, Muller's results have been confirmed by others. Moreover, it was soon learned, both for animals and for plants, that mutations can be induced not only by Roentgen rays but also by radium and by ultra-violet rays. Even more significant are the results, reported first by Goldschmidt and later by Jollos, that increase in temperature induces mutations in *Drosophila*. These experimental findings have given genetics a new lease on life.

We are justified, I think, in drawing two conclusions from this work on the induction of mutations by radiations and by heat.

In the first place, we conclude that there is no specificity in the agents which induce mutations. Roentgen, radium and ultra-violet rays and temperature are all effective though not equally so. Roentgen rays seem at present most efficacious; heat, on the other hand, judged by the failure of some workers to obtain positive results, seems least efficacious. Nevertheless, the very careful experiments made by Jollos, noted for his scrupulous methods, place the effect of temperature beyond doubt. In some respects the effect of temperature is more significant than that of the very artificial agent, Roentgen rays. The results with these rays, however, are more theatrical than those with the natural and ubiquitous agent, temperature. Even if it prove that the action of

all agents—those now known and others which may be discovered later—fundamentally affect the cell in the same way, as, for example, by causing directly or indirectly a rise in temperature, we may still argue that in the agents themselves lies no specificity.

Too often in biology we offer explanations of the responses of cells in terms of the specificity of the outside agent. Just as often, likewise, we find that the responses are due to the cells themselves which react according to their nature—that is, manifest their peculiar kinds of irritability—in a definite way, no matter what the agent eliciting the responses may be. It is thus with the unfertilized egg cell, the muscle cell or the nerve cell. All these cells attempt to respond to the experimental agent in the manner characteristic of their responses to the normal mode of stimulation. The differences found in their responses to the artificial and to the normal agents are doubtless due to the nice exactitude of the cells as reacting systems keyed with nice exactitude to the action of the normal agents and not to the crude experimental agents. On these grounds, and in the absence so far of any proof of specificity, we conclude that agents which induce mutations lack specificity. This, if it prove true, is a help, not a hindrance, to the analysis of the mechanism of Mendelian inheritance.

We may conclude, in the second place, that the mechanism for mutations which arise experimentally lies in the behavior of the chromosomes as wholes or in the behavior of units in individual chromosomes.

The chromosome theory of heredity is one of the great contributions of modern biology. A large body of cytological and experimental data indicates that the phenomena of Mendelian inheritance can be explained in terms of the behavior of the chromosomes in successive generations—that is to say, in terms of chromosomal combinations, segregations and recombinations. Furthermore, of the individual chromosomes we may state that modern work in genetics has fully sustained Roux's

('83) assumption of the serial order of the unit factors. This is likewise true of the important postulates made by Correns ('02) and by deVries ('03) concerning the unit factors in homologous chromosomes. Morgan and his school, working with *Drosophila,* have amassed a large body of data which forms the basis of the gene theory of heredity. Many other workers in many lands have been busily engaged in adding evidence in support of the chromosome theory of heredity. To-day, this theory is almost universally accepted; it is too well known to warrant here any prolonged exposition.

The cytological evidence at hand concerning the action of rays in producing mutations also indicates an effect on the chromosomes. I need merely refer to the work of Goodspeed, who has presented a cytological analysis of the behavior of the chromosomes in mutants of tobacco induced by radium and Roentgen rays. The evidence, so far meager as compared to the older cytological work supporting the chromosome theory of heredity, accumulated by other investigators falls in the same category. Hence, we may conclude that the mechanism for experimentally induced mutations lies in the chromosomes.

Is it possible, we may now ask, to explain how this chromosomal mechanism is so affected that mutations arise? In the following pages I venture to suggest an answer. This is that the behavior of the chromosomes which is responsible for mutations is not a primary one. It is itself the effect of an antecedent reaction in the cell.

II

There exists a large body of experimental work on the effects of external agents on cells. Much of it, having to do with the analysis of problems in embryology, deals with the egg cell. This work we can use as a source of evidence, since much of the support for the chromosome theory of heredity comes from the now classic cytological work—for example, the masterful contributions by Boveri on the normal egg in various stages and phases of its ontogeny.

Agents can and do affect the cytoplasm of an egg cell without manifesting any effects on its nucleus and, therefore, presumably on the chromatin thereof.

Consider, for example, the effect of fragmenting the egg. It is well known that fragments of the unfertilized eggs of many species are capable of fertilization and normal development. The presence or absence of the egg nucleus is of no consequence to development: nucleated and non-nucleated fragments develop equally well. Such an experiment, moreover, succeeds on an egg (*cf. Chaetopterus*) whose germinal vesicle has broken down and whose first maturation spindle is forming or formed. It is likewise successful on an echinid egg which having completed both maturations contains a nucleus in the so-called resting stage.

Thus the condition of the chromatin, whether on the spindle or in the intact nucleus, is without significance for the success of the experiment. It does not succeed on an egg (like that of *Asterias* or of *Cerebratulus*) in the germinal vesicle stage. In such it is necessary to await the dissolution of the germinal vesicle and the consequent appearance of the spindle; fragments now made are readily fertilizable. This indicates that a substance (or substances) diffusing into the cytoplasm with the breakdown of the germinal vesicle is necessary for the fertilization-reaction between egg-plasma and sperm; it does not mean that the chromosomes *per se* as discrete bodies affect the results of inseminating the fragments. From another angle, however, the diffusing stuffs from the germinal vesicle may be important: they may be the *modus operandi* of the exchange between chromatin and cytoplasm, thus accounting for differentiation in the cytoplasm and the impress of the chromosomal garniture on the plasma. In a similar way, it may be that with each nuclear breakdown at each stage in the whole ontogenetic process, substances metabolized by the chromosomes pass out into the plasma to order the development and to fix the genetic constitution of the embryo. But this is speculative.

Fragmentation of an egg with intact ovotid nucleus, even when most carefully done—as for example, by Tennent, Taylor and Whitaker—certainly disrupts the original organization of the intact egg. The normal nucleo-plasma relationship is upset in the fertilized nucleated fragments in which the ratio of chromatin to plasma is greater. In the larger of the fertilized non-nucleated fragments the ratio is less, because they of course contain only paternal chromatin. And yet there is no evidence that fragmentation, by careful cutting, affects the egg nucleus; chromatin does not escape. True, there is the remote possibility that fragments obtained by shaking an unfertilized egg with an intact mature nucleus might contain scattered chromatin, as Boveri in a posthumous paper felt impelled by rigorous self-criticism to suggest as a possible interpretation for one of his experiments. We should recall, however, that vigorous shaking, like strong centrifugal force on some eggs (*cf.* Conklin's work on ascidian eggs), may alter reactions in egg plasma as revealed by the egg's subsequent development. This has been clearly shown—by Boveri, Mrs. Theodor Boveri and others—especially for echinid eggs shaken soon after insemination. We may conclude that fragmentation by careful methods does not affect the egg nucleus or the chromatin thereof.

The effects on marine eggs of changes in temperature, in salinity or in hydrogen-ion concentration vary, depending on the eggs. For a given egg these effects vary before and after insemination. For the fertilized egg again there is a differential effect of the agent which runs with the mitotic cycle: the response of the fertilized egg to changes in its environment varies with stages of mitosis. But there is no evidence to indicate that this effect is primarily or alone on the nucleus or its chromosomes. On the other hand, there is evidence that the effect is primarily on the cytoplasm.

Now these changes in temperature, in salinity and in hydrogen-ion concentration of the sea-water may visibly affect the cytoplasm without demonstrable effects on the

nucleus. Where they do affect the nucleus, they do so after changes have taken place in the cytoplasm. Always, therefore, the cytoplasmic changes come first. Indeed, within optimum range, the magnitude of the cytoplasmic changes determines that of the nuclear. There is also a relationship between the duration of the exposure of the cytoplasm to these changed environmental factors and the quality and character of the cytoplasmic reaction. Again the intensity, that is, the strength of the change in the environment, determines the rate of the cytoplasmic response; and within optimum range, this rate determines the degree of the nuclear response.

Specific examples to support these statements, one may find in the literature on fertilization, experimental parthenogenesis, experiments on cell division and on development. Always changes—in some eggs most profound—take place in the cytoplasm subjected to those modifications in the surrounding sea-water named above. And always these changes precede any nuclear changes. In fertilization and in experimental parthenogenesis the response of the cytoplasm to these outside agents is most marked.

If the reader wish, he may consider this argument wholly lacking in force. On grounds of logic, then, I take the position that if he contend that these changes in the external medium affect the nucleus primarily and that the cytoplasmic response is a consequence of the nuclear, the burden of proof rests with him. Certainly, I may at least say that cytoplasmic response precedes the nuclear.

For many cells it is true that the display of mitotic activity visibly depends upon the condition of the cytoplasm. A certain complex of physical and chemical conditions in the cytoplasm is necessary for the initiation and completion of mitosis. Conklin has described the behavior of the spindle in the egg of *Sycotypus*. Here the spindle lies in very fluid cytoplasm and grows at the expense of the loss of this fluidity. Reaching a certain size, it can complete mitosis. Wilson has detailed the process of cell division in the egg of *Renilla,* in which

two nuclear divisions ensue before division of the cell body—a phenomenon deserving more study.

Egg cells have been subjected not only to changes in the temperature, in the salinity and in the hydrogen-ion concentration of the surrounding sea-water; they have also been exposed to radium, Roentgen and ultra-violet rays. One noticeable effect of such exposures is on the cytoplasm. Work on the egg of *Nereis* may be cited.

Some years ago Packard reported results of exposing eggs of *Nereis* to radium. The first visible effect is cytoplasmic. In consequence or at least in sequence to this follow modifications in nuclear behavior. Redfield especially has analyzed the cytoplasmic response displayed by this egg after exposure to various rays. Also in this same egg Just found that ultra-violet rays induce profound cytoplasmic changes which make possible abnormal nuclear behavior.

It is well known that radiations, especially radium and Roentgen rays, are most effective on cells after nuclear breakdown. An exposure to these rays which does not affect the cell while its nucleus is intact has profound effect when after breakdown of the nucleus the cell is in various stages of mitosis. It is therefore held that such radiations directly affect the chromosomes. Indeed, some workers consider—I think it is no exaggeration to say—that Roentgen rays, for example, are wholly a chromosome-affecting instrument. A moment's reflection, it seems to me, gives rise to some questioning of this position.

In the first place, radiations are not alone among agents which are more effective on cells in stages of mitosis than on those with the so-called resting nuclei. Certainly, for egg cells there is abundant evidence which indicates that paralleling the rhythm of mitosis is a rhythm of susceptibility and resistance of the cytoplasm to many and diverse experimental agents. The rhythm of nuclear division is itself parallel with the rhythm of physico-chemical and morphological changes in the cyto-

plasm. If, therefore, we argue that Roentgen or radium rays are exclusively chromosome-affecting agents, because of their greater effect during mitosis, we must then argue similarly concerning the effect of all external agents which behave likewise. It is simpler to place the action of radiation in the same class with these other experimental agents.

What is true is rather this: radiations are far nicer agents than, for example, hypo- and hyper-tonicity. Their effects are more rapid, more exact and more widespread in a given population of cells. This, in my judgment, is the chief value of radiations as experimental agents—not their so-called specific action on chromatin.

Secondly, ultra-violet rays which have feeble penetrating power act in some cases as do Roentgen or radium rays; ultra-violet then affects cells more superficially. Hence, it is not the penetrating power of the rays and so their power to reach the more deeply lying chromosomes which is responsible for their effects. Moreover, we must know for deeply penetrating rays that they produce no effects on the cytoplasm in the course of their penetration to reach the chromosomes.

Nor does the fact that in Roentgen therapy there is a difference in the susceptibility of human cells vitiate the argument. We still need to know on what this difference depends. And if we agree that this difference depends on the mitotic capacity of the cells—cells highly endowed with division and growth capacity being more susceptible —still are we far from proving that the rays are chromosome specific in their action. There is still the possibility that such division- and growth- capable cells have cytoplasms whose condition renders them more susceptible than other cells to radiation.

This consideration leads us then to the third point: namely, that materials entering the cell—gases, water, etc.—come into relationship first not with the nucleus but with the cytoplasm. We may here reason, therefore, from analogy. Consider oxygen consumption.

There was a time when biologists assumed that the nucleus is the seat of cellular oxidations. The presence of iron in the nucleus was postulated as part of the oxidation mechanism. On *a priori* grounds one would assume that oxygen entering the cell would combine with cellular constituents lying in the cytoplasm between the cell boundary and the nucleus. Now we know that this is at least nearer the truth: oxygen consumption is a function of cytoplasmic structure. The beautiful work of Warburg, in the forefront of modern physiological research, puts this beyond doubt. Moreover, Lynch was unable to demonstrate the presence of iron in the nucleus of sperm, though his method was most excellent.

What is true of oxygen is doubtless true of other substances that enter cells: they affect the cytoplasm first. In the case of agents like the radiations under discussion, which possess destructive action to a high degree we must prove that they can penetrate cells without influence on the cytoplasm lying in the path which they traverse before reaching the nucleus. It is more reasonable to assume that radiations do affect the cytoplasm.

All these considerations the reader may deem as of no great weight. They constitute in the whole merely a suggestion. No biologist to-day assumes that the nucleus can play a rôle in vital phenomena apart from the cytoplasm; both constituents of the protoplast are necessary. On the other hand, the conception of the cytoplasm as "the kitchen of the nucleus" is fraught with consequences ill to the resolution of problems of the biology of the cell. However, for the sake of analysis, though we firmly believe in the subsidiary rôle of the cytoplasm, it would seem worth while to examine this point of view: namely, that nuclear (and therefore chromosomal) behavior is secondary to that of the cytoplasm. I suggest that the cytoplasm determines nuclear behavior; the chromosomal behavior then is an expression of a more fundamental cytoplasmic activity.

Experimental cytology gives instances of aberrant behavior of the chromosomes in egg cells. This work indi-

cates that the experimental agent affects the cortical cytoplasm. The theory advanced here is that this action of the agent on the egg cortex is responsible for secondary effects on the whole egg cell. Among such effects is the aberrant behavior of the chromosomes.

III

In the early days of the modern work on genetics, the experimental analysis could not have got very far without the cytological work on the behavior of the chromosomes. In the first line of this classic period for cytology stands Boveri's work. Consider, for example, his beautiful studies on multipolar mitoses in echinid eggs.

Boveri's masterful analysis of these experiments on dispermic fertilization excites only admiration for his genius. Development depends, this work established, not on the number but on the proper combination of chromosomes. Here is a possible starting-point from which we may begin an attempt at the union, nowadays seemingly hopeless, of genetics and the physiology of development. But it is in another direction that this work of Boveri's demands attention.

A fundamental postulate of genetics is that the chromosomes of a given species differ qualitatively. Boveri's work on dispermic echinid eggs was therefore important, since it proved these qualitative differences. Normal development, he showed, depends upon the proper distribution of chromosomes to the cells derived by cleavage from the egg fertilized by two spermatozoa and therefore possessing an extra set of paternal chromosomes.

To secure dispermic or polyspermic fertilization of an echinid egg, one must first weaken it or employ heavy insemination. Heavily inseminated eggs do not all fertilize. In some cases, such eggs never fertilize. Since, however, after weakening the egg, polyspermy is rendered more easily possible, it may be assumed that where such weakening has not taken place those eggs which are fertilized by more than one sperm are weakened at the outset. Or, we may assume, that heavy insemination

itself induces or hastens a lowered resistance to supernumerary sperm on the part of some eggs among a population preponderantly monospermic. Certainly, experimental polyspermy more easily takes place in weakened eggs which show a slower response to insemination. In normally polyspermic eggs it is doubtless true that the slow reaction of the egg to insemination renders possible the entrance of supernumerary spermatozoa, but in these eggs only one spermatozoon unites with the egg nucleus; development always proceeds with the diploid number of chromosomes.

The aberrant development of the blastomeres in Boveri's experiments is due to the wrong combinations of chromosomes. But these combinations themselves depend upon the weakened conditions in the cytoplasm which make dispermy possible. In the normal egg the unimpaired cytoplasm protects against disorder of the chromosomes.

The most important work on the aberrant behavior of paternal chromosomes in cross-fertilized echinid eggs is that done by Baltzer ('70, '10). In certain crosses the paternal chromosomes fail to take part in the mitotic process. Instead they are eliminated from the spindle. Other workers have made similar observations. The possibility for cross-fertilization in all these cases is rendered greater by injuring the cytoplasm. Here again, therefore, the condition of the cytoplasm determines the behavior of the chromosomes. To induce cross-fertilization especially between widely separated species, for most eggs at least, one must impair the integrity of the eggs' ectoplasm or cortex. Such an impairment means a weakened cytoplasm. Like experimental polyspermy, cross-fertilization succeeds best after injuring the cortex of the egg.

A point here must be emphasized. Too frequently biologists speak of the incompatibility of chromosomes to account for their behavior in cross-fertilized eggs. Thus, the elimination of foreign chromosomes is held as

evidence for this. As a matter of fact, chromatin may be eliminated in *straight* fertilized eggs, as Gray has shown. Here there can be no question of the incompatibility of chromosomes in foreign cytoplasm. Rather, the chromosomes fail to take part in the ensuing mitoses because of the weakened condition of the cytoplasm previously treated—in Gray's work by hypertonic sea-water. Hertwig's work on the effect of temperature on the sperm nucleus in the echinid egg and Wilson's with ether are amenable to the same interpretation. Moreover, it could scarcely be argued that the specific sperm chromatin fails to play a part in the fertilized egg of *Rhabditis aberrans* because of its incompatibility! I have found in straight fertilized eggs of *Echinarachnius* that the *whole* egg nucleus may fail to take part in the cleavage mitosis. Such eggs are injured. The behavior of monaster eggs (M. Boveri, Th. Boveri) is a case in point. The abnormal behavior of chromosomes in these cases is clearly due to injury of the superficial cytoplasm brought about by vigorous shaking at a time after insemination when the cortex is most susceptible to experimental treatment.

The effect of radiations (referred to in the foregoing section) on straight fertilized eggs may be recalled. Here again the aberrant behavior of the chromosomes is in consequence of cytoplasmic injury or change. This injury is a definite cortical injury, according to Packard, Redfield and Just.

In all the foregoing it is safe to conclude that the injury to the cytoplasm is a cortical injury. We may therefore make the hypothesis that in cortical behavior lies the cause of the behavior of the chromosomes. Normal chromosome distribution and combinations depend upon the integrity of the cortex; their aberrant behavior is the effect of the loss of this integrity. Such behavior may manifest itself in chromosomal elimination, fragmentation, and the like.

Further, arguing from the foregoing, we may assume that the orderly normal behavior of the chromosomes depends upon cortical condition. This would mean, therefore, that chromosome-behavior is not a primary one, but rather that it is an expression of the ectoplasmic reactions.

In a sense, the normal behavior of the chromosomes is too rigidly mechanical for them to be responsible as primary agents in heredity. Such a mechanism strongly suggests some deep-seated force of which they are the expression. If we compare them to soldiers going through maneuvers, we must then assume some source of command. Their orderly behavior is not automatic but conditioned. It is as though we were to look on puppets in a show—however exact and wonderful their movements, however much we forget at times that they are puppets, soon we recall them for what they are. The chromosomes in this sense mark the reactions of the cytoplasm in which they lie. That these reactions are so far too subtle for analysis should not down us; we must not in our enamored state over chromosome behavior become blind to an alternative hypothesis.

From this point of view the gene theory is a conception too ultra-mechanistic to yield further profitable results. A conception in terms of reaction-velocity *in the cytoplasm*, difficult though it may be for attack, must be considered. And in time doubtless we can discover a mode of attack.

For me it is difficult to divorce nucleus (and chromosomes) from plasma. Nor can I conceive that even the most ardent supporter of the gene theory does so. The cell is a unit: the nucleus influences the plasma, the plasma the nucleus. The cell reacts as a whole. Sharply to divorce these two constituents of the protoplast is to make them abstractions. Furthermore, we can not isolate cells from time and space, as we all realize. If we could more properly evaluate the time-order in cell-processes, their reactions, their antecedent behavior in terms

of cause and effect, we could go farther. The analysis of the time-order in cortical reactions and response is possible. A great deal could be done by a more careful study of cortical structure in relating its changes in time.

So with space. One can not overlook the environment of cells. This is most apt here. At the outset, the development of the modern school of genetics began with the analysis of sex with special reference to its relation to the presence of a particular chromosome or chromosomes. Now surely we do not hold to the early and rigid theory of sex—especially in the light of the work by Hartmann, Herbst and others.

It is highly probable that the first effects of the environment manifest themselves on the cortex. It stands as the medium of exchange between the cell's inner and outer worlds. It is first impressed. The superficial reactions in protoplasm therefore come first. And these reactions certainly must affect the whole cell-system.

In time doubtless our biology of the cell will in all its manifold subdivisions reach the point of excellence attained by Warburg's work. Some method of attack will be devised for the analysis of the fundamental problem in cytology. This will doubtless be physiological. In the meantime, we must appreciate fully the morphological substratum of cells. Hence, this present attempt.

To many readers this discussion doubtless will appear wholly illusory and fantastic. I own that it is speculative. But I offer it as a suggestion—not at all as an antidote for the gospel of the original and ultimate dominance of the gene as the forerunner of all cellular metabolism.

We can not always go forward by blind acceptance— so dogma arises. Science does not progress thus. The present state of biological thought and work augurs some kind of generalization. In terms of structure, in terms of reactions both within and beyond its boundaries, the cortical protoplasm appears important for vital phenomena.

DIPLOID MOSAICS IN HABROBRACON

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Two kinds of mosaics reported as occasionally found in Habrobracon are haploid mosaic males from heterozygous virgin mothers and gynandromorphs or sex mosaics from mated females which may be homozygous or heterozygous. The frequency of discovery of the former has been variously estimated as one in 500 or one in 5,000 male offspring from heterozygous females, depending upon stocks used and conspicuousness of the character difference in the segregating fraternities. Gynandromorphs are about equally frequent in relation to females. Genetic evidence indicates that both of these types of mosaics develop from binucleate eggs and that in the case of gynandromorphs one of the nuclei is fertilized.

Egg binuclearity has been postulated to account for a minority of the mosaics found in Drosophila, while the great majority are accounted for by chromosome elimination in an early embryonic stage. The reverse condition obtains in Habrobracon. There have been previously reported three diploid mosaics. One female (279) from a cross of female with the recessives, ivory eyes and reduced wings, by type male, was type except that the right primary wing was reduced. It bred as a heterozygote of normal fertility producing type, ivory, reduced and ivory reduced. One female (313) from orange-eyed female by ivory reduced male was orange as expected, except that the left primary wing was reduced. It bred as an orange-ivory compound heterozygous for reduced producing orange, ivory, orange reduced and ivory reduced. One biparental male (318) from ivory reduced female by ivory male was ivory as expected, except that the left wings were both reduced. It proved completely

sterile, although matings were observed with six females which produced males only, 946.

Four new diploid mosaics have been found.[1] A type female heterozygous for cantaloup (eye color), c, for long (antennae and wings), l, and for semilong (antennae and wings), sl, was crossed with a cantaloup long male. Cantaloup and long are linked with about 10 per cent. crossing-over, while semilong segregates independently. The cross may then be expressed $Cc.Ll. Slsl \times c.l. Sl$. Besides the expected classes of offspring there appeared a mosaic female (469).

Left antenna was long; right type. Left eye was cantaloup, right was cantaloup dorsally and black ventrally. Ocelli were cantaloup. Left wings were long, right were type. There was no asymmetry in body pigmentation. Breeding test showed that the mosaic had not mated, for males only were produced—type 4, semilong 12, cantaloup long or cantaloup long semilong 8. Long can not with certainty be separated from long semilong, since they both have curved wings. Linkage is shown by the fact that males with cantaloup eyes are long, while those with black eyes are non-long.

The mosaic may be regarded as having received $C.L. sl$ from its mother and $c.l. Sl$ from its father. This instance is of especial interest because two linked recessive traits appear in the same diploid mosaic. The maternally derived chromosome bearing dominant allelomorphs to cantaloup and to long was lost in early development.

Crosses were made by Magnhild M. Torivk of females with ivory eyes, o^i, and defective R_4 wing veins, d, by males with yellow (basal segments) antennae, Y. These factors show no linkage. From one of these crosses a mosaic biparental male appeared (344). Antennae were both yellow at base but somewhat darkened, as is char-

acteristic of the heterozygote. There were 23 segments in each antenna. Left eye was ivory, right was ivory dorsally, black ventrally. Ocelli contained red pigment granules (modified ivory). Wings were type and symmetrical. Body pigment was symmetrical. The mosaic was tested by observed matings with four females but was completely sterile.

This specimen may be regarded as of composition $\frac{o^i\ d\ y}{O\ D\ Y}$. Dominant allelomorph to ivory was lost in early development affecting the eyes in part.

Crosses were made by Edward J. Wenstrup of females with orange eyes, o, and defective R_4 wing veins, d, (stock 3) by type males. Besides the expected classes, orange defective males, type females and type biparental males, there was found a mosaic biparental male (411). Each antenna had 22 segments. Eyes were black except for orange region in posterior portion of left. Median and right ocelli were black. Left was light but had some light-brown pigment (modified orange?). Wings were type, but left primary was shorter than right. Body pigment was lighter on left side of occiput and on right side of mesonotum. No breeding test was made.

This biparental male may be regarded as of genetic constitution $\frac{o\ d}{O\ D}$ in which chromosome carrying dominant allelomorph to orange was lost from embryonic region, including part of left eye and left ocellus. Factors affecting body pigment and wing length may have been lost with the same chromosome producing asymmetry in these traits.

From a cross of ivory, o^i, defective, d, (stock 17) female by orange, o, miniature, m, male, there appeared a biparental male mosaic (337). Left antenna had 22 segments, right had 21. Eyes and ocelli were orange, the latter with much red pigment. Wings and legs were all miniature on the left side; all type on the right.

Venation was non-defective. Left mesonotal pigment mark was smaller than right. No breeding test was made.

Orange and miniature are linked with about 10 per cent. crossing-over. Defective is independent. The mosaic may be regarded as $\dfrac{o^i}{o}\dfrac{M}{m}\dfrac{d}{D}$ in which chromosome bearing M and some factor for body pigment was lost from left side of thorax.

Discussion

Relative Frequency or Diploid Mosaics

Diploid mosaics in Habrobracon are evidently rare. The seven instances here reported stand in contrast to the 70 cases of haploid mosaic males from heterozygous virgin mothers and the 78 gynandromorphs or haplo-diplo-mosaics from mated females.

The conclusion should not be drawn, however, that egg binuclearity is about 21 times as frequent as somatic mitotic irregularity. Probably the former comes to light much more frequently than the latter. Binucleate eggs have a chance of being identified if fertilized or if they are produced by heterozygous mothers and undergo post-reduction. These facts eliminate the chance of finding them among the large numbers of haploid males in the studies on male biparentalism, for in this case mothers are homozygous. They should, however, be found as gynandromorphs among the females in these counts, and as mosaic males in the extensive counts from heterozygous mothers in linkage studies.

Somatic mitotic irregularity would probably fail to appear among the wasps developing from unfertilized eggs as in this case loss of a chromosome would be lethal. This eliminates the possibility of finding such irregularity among all haploid males.

The question may then be asked: Why do we not have as many mosaic females as gynandromorphs? A partial answer to this is to be found in the fact that gynandro-

morphs may on the average be more easily recognized than mosaic females. Contrast in secondary sexual traits is very striking and pertains to diverse parts of the body. Many of the mutant traits, on the other hand, affect only a limited region, such as eyes, and these are the very character differences used in the majority of crosses. Females may then be mosaic genetically, without having the difference show at all.

If we may judge from the seven diploid mosaics found, we may expect much more chromosome irregularity in biparental males than in females. Numbers of the former counted are very infrequent in comparison with the latter. Many crosses produce no biparental males at all, and in those that do the frequency is very low rarely rising above 10 per cent. of that of females. Nevertheless, we have among the seven diploid mosaics four males and three females. A somewhat larger number of mosaic females may have occurred than these figures indicate, inasmuch as biparental males may be more carefully observed than females, but this fact can not greatly affect the frequency, since the characters observed—eye color, wing size, body proportion—are very conspicuous.

It is to be presumed that irregularity in distribution of the sex-chromosome (?) is the cause of a biparental being a male. The cause of this irregularity may then affect other chromosomes as well, producing a greater tendency for mosaicism among such biparentals as are males.

Diploids or Haplo-diploids?

The question may be considered, ''Are these mosaics completely diploid or are they haplo-diploids, gynandromorphs?''

The first answer to this is that none of them shows any trace of sex-mosaicism. Such would be expected to appear inasmuch as regions affected are extensive.

The three males given breeding test (318, 344, 469) proved completely sterile. Sterility is characteristic of biparental males whereas the only two gynandromorphs

which could be induced to mate were highly fertile. (P. W. Whiting and Edward J. Wenstrup.)

With only two exceptions (P. W. Whiting) gynandromorphs have proved matroclinous in male parts, whenever source of these parts could be identified (42 cases). In these two exceptional instances, the paternal trait was dominant. Three (313, 337, 469) of these seven mosaics showed recessives derived from fathers. Such would not occur without androgenesis involving origin of male parts from supernumerary sperm nucleus or unless trait were sex-linked and gynandromorph were formed, as in Drosophila, by loss of maternal X-chromosome. An excess of recessive traits from maternal over paternal source might have been expected, since many more crosses are made with recessive females (studies of male biparentalism) than the reciprocal.

ASYMMETRY IN WILD TYPE CHARACTERS

Despite great variability, both genetic and environmental, in such traits as number of antennal segments, general body pigmentation and wing size, these traits are symmetrically developed in almost all wasps. It has been noted that haploid male mosaics and gynandromorphs show much asymmetry in these variable wild-type characters. If we except those instances in which structure was affected directly by the mutant factor so that one side of body was type while opposite was mutant and also those instances in which character was not specifically recorded, we nevertheless have a striking record of asymmetry in type traits. Of three records of antennal segment count (337, 344, 411) one (337) proved asymmetrical. Of four records of body pigment (337, 344, 411, 469) two (411, 337) proved asymmetrical. Of two records of wing size, (344, 411), one (411) proved asymmetrical. Observations were in these cases made without exact measurements, except that in case of an-

tennae, segments were counted. Such records indicate therefore striking asymmetry.

It is to be expected that chromosome elimination, just as egg binuclearity with post reduction would cause asymmetry in such type differences as are determined by multiple factors.

Correlative Gene Effect

Data are being accumulated indicating that, while in the majority of instances traits in mosaics are locally determined, there are many instances of correlative gene effect so that tissues of one genetic constitution are modified in the mosaic in the direction of the alternative trait. Ocelli of mosaics frequently show more or less of this intermediate condition. In the case of these diploid mosaics the same condition obtains. Biparental male, 344, presumably of genetic constitution, Oo^1, showed black and ivory in compound eyes, the latter little if any modified. Ocelli were, however, orange, showing distinct red pigment granules. Biparental male, 411, presumably of genetic constitution, Oo, had mixed ocelli with the lighter showing not orange but light brown pigment.

Several of these cases of "modification" may be explained by mixture of tissues of diverse genetic constitution. The matter is reserved for discussion in connection with the more extensive data in other mosaics.

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SHORTER ARTICLES AND DISCUSSION

GREEN'S STUDIES OF LINKAGE IN SIZE INHERITANCE

Dr. C. V. GREEN has published in a recent number of this journal (Nov.–Dec., 1931) an important paper dealing with the subject of size inheritance in mammals. His observations were made on a cross between two species or mice which differ in body size but produce fertile hybrids. The larger parent was a laboratory race of the house mouse, *Mus musculus;* the smaller parent was a race of *Mus bactrianus* from China. The large parent was homozygous for three recessive color mutations, non-agouti (a), brown (b) and dilution (d). The small parent bore corresponding dominant allelomorphs, agouti (A^w), black (B), and intensity (D).

The F_1 animals were heterozygous for all three color genes and were back-crossed with the large triple recessive race for the purpose of detecting possible linkage between one or more of the color genes and the larger size of the recessive parent. Such evidence was obtained. Dr. Green's paper shows careful observation and conservative deduction of conclusions. I am fully convinced of his accuracy in both regards, so far as the hypothesis which he set out to test is concerned. But there are certain considerations which seem not to have occurred to him that conceivably qualify those conclusions. These I wish briefly to present.

Green's observations show unmistakable association of larger body size and brown coat color in the back-cross generation. This is shown in the significantly greater adult body weight of both sexes and in the several bone measurements and body length, which are in general greater. To some extent but less emphatically, the dilute individuals are larger than the intense ones. Green does not claim (in this paper, though he did in an earlier one) that larger size is a characteristic of the groups of individuals which have the third recessive character introduced from the large race, namely non-agouti (a). His tables show the adult body weight to be greater in both sexes in the non-agouti animals, but the bone measurements favor the agouti animals. The evidence is thus conflicting.

I am inclined to attach greater importance to the body weights than to any other observations made by Green because they alone

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involve the entire organism and show its character at various stages of growth from birth to maturity. To be sure, bone measurements are closely correlated with general body weight, as I have shown in the case of rabbits, where the racial size differences studied were much greater than those involved in Green's mice. Green also finds a fairly high correlation between certain of the bone measurements but does not report on the correlations of body weight with bone measurements.

Green says (p. 504) "In the matter of weight, brown mice of both sexes are significantly heavier than blacks at the age of 181 days. A perusal of Table I, however, shows that this condition does not prevail at all ages, since in early life the situation is reversed, perhaps because of the initial effects of the dominant gene. In neither of the other factor pairs is there a significant difference in adult weight."

Nevertheless Green's Table I shows that in the case of the other two factor pairs recessives have a greater average adult weight than dominants in all cases except that of the dilute males which are less heavy at all ages, though their bone measurements as adults are greater than those of the corresponding dominants.

The greater *early* body weight of the dominant group is in contrast to the greater *adult* body weight of the corresponding recessive group not only in the case of the black gene but also in that of the agouti gene and of the intensity gene, except in the case of the male dilutes. I should be inclined to ascribe the greater early body size of the dominant groups not to "the initial effects of the dominant gene" but to heterosis. Each of these back-cross dominant groups is *heterozygous*, whereas the corresponding recessive group is homozygous for the gene under consideration. The heterozygous state makes for early accelerated growth, but does not necessarily affect the adult weight at all. This is made very clear in Waters' (1931) recent study of a Brahma-Leghorn poultry cross.

In adult weight Brahmas are large, Leghorns small and F_1 hybrids intermediate, but as chicks the F_1 birds are heavier than either parent breed, an obvious heterosis effect. A similar relation is seen in the relative weights of chick embryos prior to hatching, according to the observations of Byerly on a cross of White Leghorn fowl with Rhode Island Reds. See Castle and Gregory, 1931.

Also in crosses of rabbits, the F_1 hybrids are nearly or quite as large as the uncrossed large race in early life, but become intermediate or even closer to the small than to the large race, when adult. This is not due to the "initial effect of the dominant genes" because all the four dominant genes were present in the large race in the rabbit cross studied, yet rabbits *heterozygous* for these genes showed accelerated early growth but intermediate adult size.

The same types of growth curves are found in respect to all three pairs of genes among Green's back-cross mice. An accelerated early growth occurs in the heterozygous group (agoutis of both sexes, blacks of both sexes, and intense pigmented mice of the female sex) but the adult weight in all these groups is less than that of the corresponding homozygous recessive group. Consequently the growth curves of the heterozygotes and of the homozygotes of each factor pair cross, in the case of the agouti gene subsequent to the age of 61 days, in the case of the black gene subsequent to the age of 31 days, and in the case of the intensity gene for females only between the ages of one and eleven days. It seems fair to assume that a similar phenomenon occurs in each case, an accelerated early growth of the heterozygote class, which nevertheless is genetically the group of smaller body size as shown by its adult weight.

If we adopt this interpretation, then we shall accept Green's conclusion that there is genetic linkage between large body size and the recessive genes brown and dilution, and we shall extend the conclusion to take in the recessive gene non-agouti also. All three recessive genes were introduced into the cross in association with large body size. They also emerge in the back-cross in the same association. Does this mean that there are three separate genes (or groups of genes) for large body size located in the three chromosomes which bear respectively the a, b, and i genes? Not necessarily. I certainly should have so interpreted it, had I found such association to occur in the case of the four color genes involved in the rabbit size cross which I studied, but no evidence of it could there be found. Wherein then do the two cases differ? The mouse cross is not a cross of breeds, as was that of the rabbits, but a species cross. Gates (1926) has studied a cross of domesticated strains of these same two species using the Japanese waltzing mouse as a representative of *Mus bactrianus* (*Wagneri*). In a cross between pink-eyed dilute brown

house mice (related to the dilute brown race used by Green, both being derivatives of Little's dilute browns) and black-and-white Japanese waltzing mice, five independent genes were involved. The house mouse parent furnished three of the five recessives, namely pink-eye, brown and dilution, including two of the three recessives used by Green; the Japanese parent furnished the other two recessives, namely piebald and waltzing. In the F_2 and back-cross generations these five genes were found to keep together in their original associations in excess of the proportions demanded by random assortment, the deviation from expectation ranging from 0.1 to 8.2 times that due to chance.

In harmony with the observations of Gates, we should expect that in the cross studied by Green the recessive genes, a, b, and d, would be found together in excess of their chance frequencies. It seems clear from Green's Table I that a slightly larger body weight is also associated with this same combination of recessive genes. This does not necessitate the conclusion that larger body size is determined by genes located in the same chromosomes as a, b, and d, but only that *whatever* causes a, b, and d to go together, causes larger body size to go with them. That something is not ordinary genetic linkage (location in the same chromosome) as proved by the observations of Gates. It must be something connected with all three chromosomes and also a factor in size determination. What known structure has all these relations? Only the egg and sperm plasma which are extra-chromosomal constituents of the germ-cells. I have been inclined to look in that direction for the genetic basis of adult body size by the results of studies on rabbit crosses and rabbit embryology reported elsewhere, but I have hesitated to put forward an hypothesis so at variance with the currently accepted theory of the gene. Green's results distinctly favor such an interpretation rather than that of ordinary chromosomal linkage.

Gregory and I have shown that differences in general body size in rabbits are determined primarily by differences in rate of development of the fertilized egg. Though the eggs of different races are at fertilization indistinguishable in size and appearance, those of a large race segment more rapidly and subsequently increase in bulk faster than eggs of a small race. Differentiation however occurs at substantially the same rate in both. Consequently, at birth, large race rabbits are larger and they continue to grow faster after birth than do rabbits of a small race.

This difference in rate of development can not be referred to the action of genes lying in a single chromosome, as is shown by the manner of its inheritance, which is blending and requires a multiple factor scheme, if explained on the basis of chromosomal genes. I have been unable to find evidence of the existence of such genes in four of the twenty-two chromosome pairs of the rabbit, though I have made a search for it by the method employed by Green. I suspect that the difference in our results is due to the difference in our materials. The apparent linkage demonstrated by Green, I am inclined to think, is due to the same peculiarity of his material, which in Gates' experiments caused genes known to be located in five different chromosome pairs to segregates oftener than in their random proportions in the same association which they had in the parent species.

That chromosomal genes can influence the size or form of particular parts of the organism has been amply demonstrated. I am quite willing to concede all that Lindstrom claims in this regard. He has demonstrated the existence of linkage between genes governing the size and color respectively of the seeds of maize and of the fruits of the tomato. Among animals a case equally clear was demonstrated by Gates, the linkage between short ear and dilute pigmentation in mice. The entire short-ear effect is here determined by a single gene. The case is very different in the inheritance of ear-length in rabbits. Here the ear-length is a function of general body size, with which it is closely correlated, and it is doubtless determined by the same causes. The same developmental rate which produces a large rabbit will produce a long-eared one, and the developmental rate which produces a small rabbit will produce a short-eared one. What we must look for in this case is a genetic basis for rate of development. Such a basis in my opinion is more likely to be found in extra-chromosomal components of the germ-cell, since linkage can not as yet be demonstrated with chromosomal genes.

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GENETIC LINKAGE IN SIZE INHERITANCE— A REPLY

DR. W. E. CASTLE has kindly submitted to me the manuscript of his alternative explanation of my observations (1931a) on size inheritance in a mouse species cross. For the association in heredity between factors productive of a large size in several quantitative characters and a qualitative character, brown coat color, which I interpreted as genetic linkage, he offers a quite different explanation. His interpretation seems to be partly dependent on some misconceptions of my data, points which I evidently failed to make clear.

Castle states: ''Green's observations show unmistakable association of larger body size and brown coat color in the back cross generation. This is shown in the significantly greater adult body weight of both sexes and in the several bone measurements and body length.'' The situation, as I tried to make clear, was that brown coat color was associated with larger size in several size characters,—not in *the* several. For example, skull length or width gave no clear evidence of being so associated while femur and tibia length did. I considered these differences in behavior to further indicate that not all size factors are general.

There was no intention of claiming in an earlier paper that

"larger size is a characteristic of the groups of individuals which have the third recessive character introduced from the larger race, namely non-agouti (a)." I believe I stated merely that there were indications of linkage of larger size in some of the quantitative characters and the three recessive qualitative characters studied. It was realized that linkage was by no means demonstrated, hence the repetition of the experiment under more uniform conditions.

In the Ann Arbor work (the work reported on in previous papers) the mice were born over a considerable length of time and the back cross was made in two ways, thus reducing the number in each type. In the Bar Harbor work (reported on in "Linkage in Size Inheritance") all the animals were born within a period of less than six months and were all from one type of mating. For these reasons among others it is probable that the Bar Harbor material provided the more uniform data and should be considered separately. It is from these data that I considered linkage to have been demonstrated.

It is true that I found a fairly high correlation between certain of the bone measurements but significantly higher correlations between some pairs (*e.g.*, femur—tibia) than others (*e.g.*, skull—femur). In another paper (1931) I reported on correlations of body weight with bone measurements in the pure *musculus* race. Although I believe adult weight, body length and bone lengths all to be manifestations of general size, yet they are not identical manifestations since each is influenced by local as well as general factors. The paper just cited gives reasons for this view-point.

Whether the superiority in weight at early ages of blacks over browns can best be attributed to "the initial effects of the dominant gene" as I did or to the heterozygous condition as Castle did seems to me to be largely dependent on our respective views as to the nature of heterosis. I have been inclined to adopt the purely Mendelian interpretation of inbreeding as developed largely by Shull, East and Jones. Following this, heterosis is not attributed to the heterozygous condition *per se* but to the breaking up of some of the recessive factor pairs by the introduction of dominant allelomorphs. So whether we attribute the early larger size of the heterozygous blacks to heterozygosity or to their possession of the dominant gene seems to be chiefly a matter of terminology.

The explanation which Castle substitutes for my interpretation of linkage is summarized in his statement:

> In harmony with the observations of Gates, we should expect that in the cross studied by Green the recessive genes, a, b and d, would be found together in excess of their chance frequencies. It seems clear from Green's Table I that a slightly larger body weight is also associated with this same combination of recessive genes. This does not necessitate the conclusion that larger body size is determined by genes located in the same chromosomes as a, b, and d, but only that *whatever* causes a, b, and d to go together, causes larger body size to go with them. That something is not ordinary genetic linkage (location in the same chromosome) as proved by the observations of Gates. It must be something connected with all three chromosomes and also a factor in size determination. What known structure has all these relations? Only the egg and sperm plasma which are extra chromosomal constituents of the germ cells.

This non-chromosomal interpretation seems to rest heavily on two assumptions: (1) That the hybrids in my experiment exhibit "association systems" of chromosomes such as Gates found, and (2) that since Castle could detect no evidence of genetic linkage in his rabbit cross (1929) which involved two of my three color genes some other explanation may be in order for the mice.

As I pointed out in a preliminary communication (1930) I was unable to detect any such "association system" of chromosomes as Gates found. A complete tabulation of the total back cross and F_2 generations (the great majority of the latter from a tumor inheritance study) of the *musculus* (dba) and *bactrianus* (DBA[w]) hybrids has just been made. The figures given on p. 90 show clearly that the parental genes exhibited no tendency to stay together but showed free assortment.

These figures include depleted as well as undepleted litters but separate tabulations have shown that no selective post-natal mortality exists. In the back cross the triple recessives show only an insignificant excess over the expected while on the other hand the triple dominants (*bactrianus* association) show even a slight decrease.

Were Castle's explanation correct in that whatever causes the three recessive color characters to go together (assuming that something does) also causes larger size to go with them, we should expect to find triple recessive mice larger than mice with the gene for brown in other combinations. Were my contention correct that the larger size of brown mice is due to size factors

	Total back cross		Total F$_2$	
	Observed	Expected	Observed	Expected
Blk. Ag. (DBA^w)	127	136.2 ± 7.4	203	204.7
dil. Blk. Ag. (dBA^w)	146	136.2 ± 7.4	70	68.2
Br. Ag. (DbA^w)	141	136.2 ± 7.4	67	68.2
Black (DBa)	136	136.2 ± 7.4	74	68.2
dil. Blk. (dBa)	110	136.2 ± 7.4	20	22.7
Brown (Dba)	132	136.2 ± 7.4	20	22.7
dil. Br. Ag. (dbA^w)	141	136.2 ± 7.4	24	22.7
dilute brown (dba)	156	136.2 ± 7.4	7	7.6
Total	1,089	1,089.6	485	485.0

linked with the gene for brown no marked differences should be encountered. If anything, the triple recessives should tend to be slightly smaller because of the piling up of recessive genes which usually are more deleterious than dominants. The following figures show that in the four quantitative characters in which significant differences were found between browns and blacks only, the triple recessives exceeded all browns in but two out of the eight contrasted pairs.

COMPARISON OF TRIPLE RECESSIVES (*Musculus* ASSOCIATION) WITH ALL BROWNS (1931a DATA)

Character		Dilute brown non-agoutis		All brown mice	
		No.	Mean	No.	Mean
Humerus length	♂ ♂	15	11.02 mm	72	11.10 mm
	♀ ♀	23	10.67 mm	77	10.74 mm
Femur length	♂ ♂	14	14.36 mm	71	14.39 mm
	♀ ♀	23	14.23 mm	76	14.24 mm
Tibia length	♂ ♂	15	16.25 mm	72	16.30 mm
	♀ ♀	23	16.03 mm	77	16.02 mm
Weight (181st day)	♂ ♂	15	22.2 grams	72	22.8 grams
	♀ ♀	23	20.8 grams	78	20.4 grams

Although Castle's rabbit cross involved two of the three genes in my mouse cross, nevertheless it was the third gene, b, which gave clearest evidence for linkage with size factors. It is quite probable, moreover, that a chromosome with a certain gene for

color might bear demonstrable size genes in one form but not in another. The chromosome with the gene for brown, it will be recalled, bears the gene for albinism in rabbits but not in mice.

A reconsideration of the data leads me still to feel that the explanation making use of genetic linkage is better borne out by the facts and involves less speculation than Castle's alternative explanation.

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TO DETERMINE GENETICAL RATIOS WHEN SELFING ORGANISMS HETEROZYGOUS FOR TWO OR MORE FACTORS

In working out the number and constitution of all possible phenotypes and genotypes, when mating individuals heterozygous for the same factors, I have found the application of the principle of the expanded binomial, as given below, more readily understood and applied by the undergraduate student than the $3 + 1$ or the $3/4 \times 3/4 \times 1/4$, etc., methods as usually given in text-books on genetics. It also greatly reduces the number of calculations necessary for determining ratios, phenotypes and genotypes by means of the checkerboard method. Furthermore, the necessity of determining the exact constitution of all possible gametes, always a stumbling block for the beginning student, is eliminated.

I am venturing to outline here the method, as we have used it for some years in undergraduate classes, with the thought that it may prove helpful to other teachers of genetics.

A. In general.
    1. An allelomorphic pair is represented by the binomial $a + b$.

2. In the bionomial "a" = 3 and is dominant, "b" = 1 and is recessive.
   a : b as 3 : 1.

B. Procedure.

1. To determine the phenotypes.

   a. The number of allelomorphic pairs involved gives the power to which the binomial is to be raised. For example, AaBbCc × AaBbCc = $(a+b)^3 = a^3 + 3a^2b + 3ab^2 + b^3$.

   b. The sum of the coefficients of the expanded binomial gives the number of phenotypes $-1 + 3 + 3 + 1 = 8$ phenotypes.

   c. For any term in the expanded binomial.

      (1) The coefficient indicates the number of phenotypes,

      (2) 3 raised to the power of the "a" exponent gives the number of individuals in each phenotype,

      (3) An exponent gives the number of dominants or recessives in each phenotype.

      For examples $3a^2b = (1)$ 3 phenotypes (2) 9 individuals in each (3) 2 dominants and 1 recessive.

   d. Application, assuming 4 allelomorphic pairs.

      $(a + b)^4 = a^4 + 4a^3b + 6a^2b^2 + 4ab^3 + b^4$.

      $1 + 4 + 6 + 4 + 1 = 16$ phenotypes.

      a^4 represents 1 phenotype of 81 (a^4) individuals each having 4 dominants, $a^4 = 81$ ABCD.

      $4a^3b$ represents 4 phenotypes of 27 (a^3) individuals each, each phenotype having 3 dominants and 1 recessive,

      $4a^3b = 27$ ABCd + 27 ABcD + 27 AbCD + 27 aBCD.

      Similarly

      $6a^2b^2 = 9$ ABcd + 9 AbCd + 9 AbcD + 9 abCD + 9 aBcD + 9 aBCd,

      $4ab^3 = 3$ Abcd + 3 aBcd + 3 abCd + 3 abcD,

      $b^4 = 1$ abcd.

2. To determine the genotypes.

   a. In each phenotype there is one and only one individual that is homozygous for all the factors concerned. Always let the genotypes for each phenotype begin with this homozygote.

   b. The number of individuals in any genotype not purely homozygous is equivalent to 2 raised to the power of the number of heterozygous dominants. Thus there are always 2 individuals when one factor is heterozygous, 4 individuals when two factors are heterozygous and so on.

   c. To make sure of a full representation of genotypes, it should be noted that in each phenotype the numbers of the different combinations in each successive group of genotypes correspond to the coefficients of the binominal raised to the power of the number of dominants in the phenotype. For example the numbers of the different combinations in the successive groups of genotypes in the heterozygous phenotype ABC are 1, 3, 3, 1, the coefficients of $(a+b)^3$.

   d. Application

a⁴	4a³b	6a²b²	4ab³	b⁴
81ABCD	*27ABCd*	*9ABcd*	*3Abcd*	*abcd*
AABBCCDD —1	AABBCCdd —1	AABBccdd —1	AAbbccdd —1	aabbccdd
2AABBCCDd ⎱	2AABBCcdd ⎱	2AABbccdd ⎱	2Aabbccdd	
2AABBCcDD ⎰ —4	2AABbCCdd ⎰ —3	2AaBBccdd ⎰ —2		
2AABbCCDD	2AaBBCCdd	4AaBbccdd —1	*3aBcd*	
2AaBBCCDD	4AABbCcdd		aaBBccdd	
4AABBCcDd	4AaBBCcdd ⎱	*9AbcD*	2aaBbccdd	
4AABbCCDd	4AaBbCCdd ⎰ —3	AAbbccDD —1		
4AABbCcDD	8AaBbCcdd —1	2AAbbccDd ⎱	*3abCd*	
4AaBBCCDd ⎱ —6		2AabbccDD ⎰ —2	aabbCCdd	
4AaBBCcDD	*27ABcD* etc.	4AabbccDd —1	2aabbCcdd	
4AaBbCCDD ⎰	AABBccDD —1			
8AABbCcDd	2AABBccDd ⎱	*9abCD* etc.	*3abcD*	
8AaBBCcDd	2AABBccDD ⎰ —3	aabbCCDD —1	aabbccDD	
8AaBbCCDd ⎰ —4	2AaBBccDD	2aabbCcDD ⎱	2aabbccDd	
8AaBbCcDD	4AABbccDd ⎱	2aabbCCDd ⎰ —2		
16AaBbCcDd —1	4AaBBccDd ⎰ —3	4aabbCcDd —1		
	4AaBbccDD			
	8AaBbccDd —1			

The points to bear in mind do not lend themselves to simple statements and seem rather formidable in the first reading. When once applied, however, the process becomes thereafter very simple for any number of allelomorphic pairs. The statement has been written out very fully in the interest of the undergraduate, not for the expert.

<div style="text-align:right">Margaret C. Ferguson</div>

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DOMINANT EYE-COLORS IN DROSOPHILA

Dominant eye-color mutations that have been produced by x-ray and radium irradiation of *Drosophila* have been found to be associated with some type of chromosomal aberration (Weinstein, 1928, Muller, 1930, Oliver, 1930, and Hanson and Winkleman, 1929). The eye-color is usually non-homogeneous and eversporting. In the fall of 1930, additional dominant eye-colors, which the author obtained by x-raying, were examined genetically and cytologically for the purpose of finding the possible relationships between the chromosome abnormality and the expression of the dominant eye-color.

Normal males were given an x-ray dosage of approximately 4,000 r units (52 kilovolts, 5 milliamperes, 20 cm distance from tube, 1 mm thickness of aluminum, and exposure time of 2.5 hours). Offspring of treated males mated to untreated attached-X yellow females were examined for departures from the

normal type. Seven dominant eye-color mutations were found among 20,929 F_1 individuals.

Two of the dominant eye-color mutations are homogeneous and stable. The first, Salmon, is associated with a mutual translocation involving approximately half of the second and half of the third chromosome. Cross-over classes furnish strong evidence that the eye-color mutation is located exactly at the point of breakage of one of the involved chromosomes. Cytological examination of dividing oogonial cells shows that both arms of the composite second and third chromosomes exhibit a strong tendency to pair with their homologues in the normal set of autosomes.

The homogeneous eye-color, Henna, a brownish-red, appeared in a fly carrying a translocation from the third to the second chromosome. However, the mutation proved to be entirely independent of the translocation, and behaved as if it were a gene mutation located near hairy on the third chromosome. It is believed that Henna is the first dominant eye-color mutation in *Drosophila* reported as definitely due to a gene mutation.

With the five non-homogeneous or eversporting eye-colors, called Dilute eyes, the normal red color appears diluted in some of the ommatidia of the compound eye. Cream-eye, obtained by exposure to radium (Hanson and Winkleman, 1929), is included in the study of the Dilute eye-colors, since it shows peculiarities similar to those observed with the "Dilutes" produced with x-rays. The eye has a cream color only when the individual is homozygous or hemizygous for vermilion at the same time that it carries the dominant eye-color (Dilute$_1$).

Usually all the ommatidia are affected in Dilute$_1$ and Dilute$_2$ and the eye is of a uniformly diluted red color. (The color of Dilute$_1$ has been made the subject of a preliminary quantitative study by spectrographic methods, E. A. and L. C. Van Atta, 1931.) Both eye-colors involve the same type of inversion in the second chromosome. A comparison of the cross-over classes and cytological configurations leads to the conclusion that the left arm of the second chromosome has become transferred to the end of the right arm, making a rearranged chromosome with a terminal rather than a median spindle fiber attachment. The Dilute$_3$ eye has a characteristically mottled appearance and is associated with an inversion in the right arm of the second chromosome. In Dilute$_4$ and Dilute$_5$ only a few of the omma-

tidia of the eye are affected, and non-reciprocal translocations between the second and third chromosomes are present. There are inversions in the second chromosome of Dilute$_6$, with small translocations from the second to the third and fourth chromosomes. Frequent non-disjunction results in the presence of three fourth chromosomes in oogonial cells. Several types of hyperploid individuals survive.

Results from tests with Bridges' counterbalanced stock of the Pale-translocation show that all the non-homogeneous eye-color mutations are located in the extreme right end of the second chromosome. The expression of the dominant eye-color is suppressed in all cases by the normal allelomorphs in the homologous second chromosome and the translocated section of the Pale stock which includes the loci of plexus and speck. That the Dilute eye-colors form an allelomorphic series in this region seems still more probable from the fact that there are no viable combinations of any of the Dilute eye-colors. This was shown by matings of Dilute Curly flies using all possible combinations of Dilute eyes.

The findings for Salmon and Henna have demonstrated that the dominant mutations in eye-color are not necessarily associated with eversporting behavior or chromosomal aberration. However, if the eversporting tendency is present, it is accompanied by some sort of chromosomal rearrangement, as seen in the Dilute eyes. These have all resulted from mutation in the extreme right end of the second chromosome at a locus that is evidently especially susceptible to mutations of this type.

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ON CERTAIN FEEDING HABITS OF THE SEA–URCHIN ARBACIA

The sea-urchin, *Arbacia punctulata*, is generally regarded as an omnivorous scavenger, evidence of which is seen in its fecal detritus. It feeds for the most part on the remains of animals and plants that collect on the sea-bottom. But it is not always limited to so tame a diet. Occasionally, it rises in its quest for food to almost sportsmanlike activities.

If a number of sea-urchins are put in an aquarium in which there is a goodly supply of mummichugs, *Fundulus heteroclitus*,

one or more of these fishes may be caught and eaten by the sea-urchins. The capture usually takes place at night, and the prey is almost always a partly spent fish. I doubt if a fully vigorous Fundulus is ever taken by a sea-urchin. I have never witnessed the first steps in the capture. What I have seen, on first arrival, is a fish, usually a small one, whose head or tail is largely covered by the sea-urchin. The spines and ambulacral feet of the captor are used to hold the prey against either the bottom or the sides of the aquarium, while the jaws of the sea-urchin are vigorously plied on the flesh of the fish. Not infrequently, a fish once caught is beset by a second sea-urchin and the two together complete the demolition of the Fundulus, as shown in the accompanying figure. The fish, when caught, often offers some resistance, but
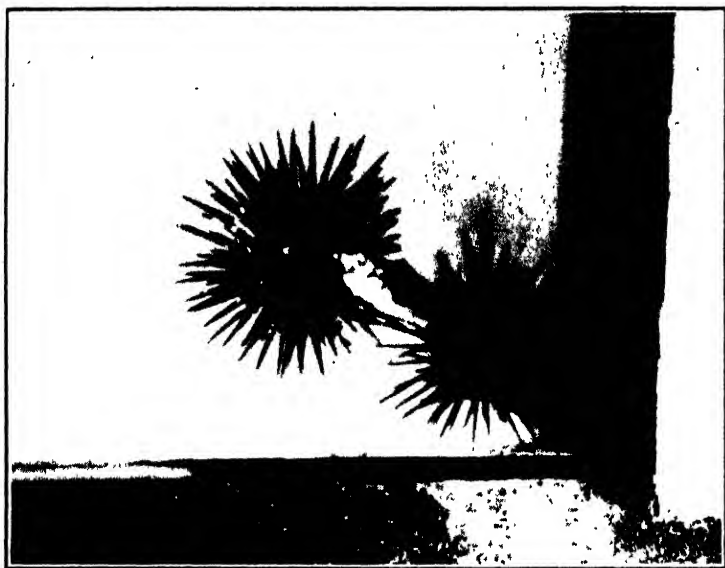
Glass wall of an aquarium against which a Fundulus is being held by two sea-urchins, *Arbacia punctulata,* which are devouring the fish. Photograph by Dr. E. C. Cole.

it soon succumbs to the sea-urchin as though it had been poisoned by its captor. However, I have no direct evidence that the sea-urchin can exert such influences. The main point that this note is intended to record is the capture of live fish by the sea-urchin, *Arbacia punctulata.*

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THE

AMERICAN NATURALIST

THE AMERICAN SOCIETY OF NATURALISTS

SEX–INHERITANCE AND SEX–DETERMINATION[1]

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In what follows, sex will be considered as implying the existence of certain demonstrable structural and functional differences. These differences distinguish complementary adaptations to needs imposed by the occurrence of gametic union, and by the increasing length of an embryonic period for which provision must be made by the parental or (in angiosperms) the grandparental generation. In some current biological discussions sex is understood more broadly than as here defined. Reference is unnecessary to more weird conceptions entertained by the uninformed—say, by post-war novelists or by literary M.D.'s.

Sexual differentiation has evidently developed independently in many distinct lines of descent. The algae, particularly the Isokontae, furnish the most extensive evidence at hand of the course of its evolution. As yet, however, the algae have revealed little regarding the determination and inheritance of sexual characters. So far as plants are concerned, it is necessary to turn to two other groups—bryophytes and angiosperms—for any extensive light upon these questions.

Any genetic analysis of sex in angiosperms must deal almost exclusively with characters of the so-called

asexual generation, since those of the much-reduced hap-
loid "sexual" generation have yet afforded little mate-
rial for genetic study. To speak of sexual characters in
an asexual generation is paradoxical; but the paradox
inheres in the terminology, not in the facts. The diploid
sporophyte helps through various devices to effect the
union of gametes produced by the filial gametophytes,
and to provide for the shelter and nutrition of the em-
bryonic grandfilial sporophyte; and such devices are
sexual characters under any usable definition of the
term.

This differentiation in the sporophytic generation is
very different in its phylogenetic implications from sex-
ual differentiation in any metazoon. Functionally, how-
ever, the same ends are subserved; and, somewhat re-
markably, the genetic mechanisms that govern in the two
cases are strikingly similar.

Many pages of discussion of the present and related
problems would not have burdened the pages of scientific
journals had their writers appreciated two fairly obvious
facts: first, that the potentiality for the production of
any character that an organism under any possible con-
ditions can manifest must be represented in its heredi-
tary constitution; and second, that this hereditary consti-
tution provides *only* potentialities, whose expression or
non-expression depends upon the concurrence of environ-
mental factors.

The question then presents itself: What potentialities
for sexual differentiation exist in an angiosperm? In a
large majority of species, each plant, under anything
approaching ordinary environmental conditions, regu-
larly produces both stamens and pistils—as well as,
often, intersexual structures. For such bisexual organ-
isms the answer to the question is, clearly, that both
male and female potentialities are represented in the
hereditary substance. On the other hand, in a minority
of species some plants produce only or chiefly staminate
flowers, other plants only or chiefly pistillate flowers. It

is mainly with these dioecious species that discussions of sex-determination in angiosperms have dealt.

In most instances in which the floral structures of dioecious, or so-called dioecious, angiosperms have been extensively studied, it has been found that a pistillate plant now and then bears a staminate or bisexual flower and that a staminate plant occasionally produces a pistillate or bisexual flower. Sometimes intermediately bisexual plants occur in addition to those which are strictly or chiefly male or female. Such conditions characterize, for example, dioecious species of nettle, hop, hemp and spinach. The closest approach to an absolute separation of sexual characters seems to be shown in *Lychnis dioica*, *L. alba* and *Bryonia dioica*. But even for *Bryonia*, there is one report of the occurrence of occasional staminate flowers on a female plant. In both dioecious species of *Lychnis* hermaphroditic plants are described which are shown, in one case by genetic, in the other by cytological, evidence to be modified males. In both species, too, it has long been known that infection by the anther smut induces the development of stamens by a female plant. The evidence, therefore, is overwhelming that in dioecious as in hermaphroditic angiosperms each plant possesses both male and female potentialities. The possibility of course remains, though present evidence opposes it, that strains or even species may be found with more limited genetic capacities.

It is generally agreed that nothing points directly to specific genes responsible for sexual potentialities. But these must be represented by some basis or bases in the hereditary mechanism. Anglicizing a term already much used, such bases may be termed, respectively, male- and female-potency factors. There is no reason to suspect that these factors are borne by X and Y chromosomes, even in those species in which such allosomes are recognized. Since every plant is endowed with male and female potentialities, the factors basic thereto must be so located that for each the plant is homozygous. This

fact excludes from consideration the Y chromosome, and, though perhaps not so certainly, the X; moreover, as will appear, the X–Y mechanism is otherwise especially concerned. So it may be held as probable that the sex-potency factors in all angiosperms are connected with one or more pairs of autosomes.

A further explanation is needed for the one-sided type of sex expression found in dioecious species. Here, evidently, factors are involved which, superposed as it were upon those just mentioned, favor the appearance of one set of sexual characters and tend to inhibit that of the other set. Factors of this superposed set have been referred to as sex-*determining*. However, since this term may irk some of our colleagues who are strongly impressed with the determining effects of the environment, it is more discreet to speak of male and female *tendencies*. An important contrast appears between sex-potency and sex-tendency factors; namely, that, whereas both (or both sets) of the former exist, each in a homozygous condition, in every plant, on the other hand, with respect to the factors for sex tendencies a condition of heterozygosis must prevail in one sex.

The recognition of a distinction between sex potencies and sex tendencies in angiosperms is due to Correns. The former he considers as represented by gene-complexes, A and G; the latter, by genes or gene-complexes, α' and γ'.

Omitting questionable cases, X and Y chromosomes are known in some 45 species, representing 19 genera, of dioecious angiosperms. The distribution of these chromosomes parallels that of the sex tendencies. The known facts fit an assumption that the female-tendency factor is located on the X chromosome, the male-tendency factor on the Y. It is quite possible, to be sure, that angiosperms may be found in which the female rather than the male is heterozygous with respect to these factors. That this is the condition in certain dioecious strawberries is strongly indicated by experimental and cytolog-

ical studies.   In 26 dioecious species, belonging to 22 genera, the search for unlike chromosome pairs has been unsuccessful.   Since the study has been limited for the most part to the microspore mother cells, it may be that the females of some of these species possess an X–Y pair.

Should species be found with an X and no Y (a condition not yet definitely known for an angiosperm), the assumption just noted will require modification, at least for those species, by supposing that the male- (or female-) tendency factor is borne on an autosome.   Such a conception would be very close to that advanced by Bridges for *Drosophila,* with the added feature of a recognition of the distinction between sex potencies and sex tendencies.

It may not be comely for a botanist to express opinions bearing upon conditions in animals.   However, since zoological writers have shown neither undue modesty nor excessive caution in treating of botanical phenomena, one suggestion will be ventured.   Correns' analysis has not found favor among animal geneticists.   But wherever hermaphroditism, intersexuality or sex-reversal occurs—and these are now recognized as wide-spread phenomena in several metazoan phyla—the potentialities for the production of the characters of both sexes must reside in each individual.   The more or less sharp differentiation of male and female individuals, the genetics of sex and the occurrence of the X–Y chromosome mechanism, all parallel to conditions noted in angiosperms, evidence the presence of sex tendencies superposed upon a duplex set of potentialities.

Sex expression in angiosperms as in other organisms is of course influenced by many genetic factors apart from those mentioned.   These others, diverse though they be, may be grouped together for present purposes as sex-*influencing* factors.   Among them are those which affect, very differently in different species, the numbers, succession and arrangement of stamens and pistils; or which bring about such modifications of strict hermaph-

roditism as monoecism, andromonoecism and gynomonoecism; or conditions intermediate between hermaphroditism and dioecism, like andro- and gynodioecism. The class would include those factors experimentally studied in maize, which influence the proportions, distribution and functioning of staminate and pistillate flowers.

Admittedly, it may not always be easy to distinguish between factors of this class and the sex-tendency factors or mutants thereof. For example, Shull concludes that the appearance of hermaphroditic *Lychnis* plants, shown to be modified males, results from a mutation of the male-tendency factor; but the very comparable occurrence of intersexes in *Drosophila simulans* is found by Sturtevant to be caused by a recessive gene located on an autosome. As another illustration, the action of certain of the factors in maize just mentioned may result in the appearance of purely female plants, thus simulating the effect of a female-tendency factor.

Environmental factors likewise produce effects comparable with those of the sex-tendency factors, inhibiting the expression of one set of sexual characters and favoring the expression of the characters of the opposite sex. An environmental factor, as nutrient or light, may thus partially or completely reverse the effects produced in the environment usual for the species by the genetic sex-tendency factors. This possibility is abundantly shown by the work of Schaffner among many others. Environmental factors also often produce effects parallel to those of the sex-influencing factors; but never anything of the order of the effects of the sex-potency factors. These latter endow the plant with both sets of sexual possibilities, and the possibilities remain unchanged, however their expression may be encouraged or discouraged by other factors genetic or external.

In considering sexual conditions in bryophytes, it must be remembered that the plants here in question belong to a haploid generation. This generation corresponds,

so far as there is correspondence, to the angiosperm gametophyte, regarding whose mechanism of sex-determination nothing is known. Another important consideration is that any relationship between bryophytes and angiosperms is at best extremely remote; possibly, indeed, they are climax members of distinct evolutionary series derived from separate protistan ancestors. It would not be surprising, then, should the mechanism of sex-determination prove to be radically different in the two groups. It would be less surprising, indeed, than was the finding of a similarity in this regard between angiosperms and insects.

The bryophytes, to be sure, show a distribution of sexual characters superficially like that found in angiosperms. Both groups include hermaphroditic and dioecious species. Whereas a majority of angiosperms are hermaphroditic, among bryophytes, to judge from taxonomic descriptions, a majority, though not an overwhelming one, are dioecious. But with respect to this point, more or less casual field and herbarium observations of limited numbers of plants must be checked by extensive observation and experiment.

Numerous sex-influencing factors are present in bryophytes, as shown by the fact, among others, of the occurrence of specific differences in the distribution of sexual organs. At least seven distinct racial characters are now known in Sphaerocarpos, each of which involves the structure or functioning, or both, of sexual organs. Environmental factors also produce comparable effects. The appearance of the sexual structures of *Marchantia* can be inhibited by conditions of illumination. Nutritive conditions are shown to determine, in some measure, the production of male or of female organs by *Funaria*. But environmental conditions have not been found able to reverse the sex expression of a dioecious bryophyte, as they can reverse that of a dioecious angiosperm.

Extensively studied thus far are a few dioecious mosses, including those involved in the classic experi-

ments of the Marchals; and a few dioecious hepatics, among them the familiar *Marchantia polymorpha* and two species of *Sphaerocarpos*. These studies agree in showing that strict unisexuality exists; that is, any haploid plant of such a species is destined from the spore to be either male or female, and no change in conditions, applied at any stage, has induced intersexuality or sex-reversal. Beside this fact must be placed another, namely, that a single genus, such as *Marchantia,* may include hermaphroditic and strictly dioecious species. There are suggestions of the possible existence of an intermediate condition—one of potential bisexuality combined with a tendency toward dioecism. But in no species thoroughly studied has this condition been found; and the possibility must for the present be ignored.

In at least five dioecious hepatics, X and Y chromosomes are certainly recognized; one X being present in the female, one Y in the male. In others, including *Marchantia,* and in dioecious mosses in general, no such allosomes have been distinguished. In these latter cases, nevertheless, the phenomena of sex expression are the same as in species known to possess sex chromosomes.

Polyploid gametophytes of normally dioecious mosses have been obtained by experimental methods—most extensively by the Marchals, Schweizer and Wettstein. A diploid gametophyte containing two maternal genoms is female; one with two paternal genoms is male. A diploid possessing one maternal and one paternal genom, or a tetraploid with two maternal and two paternal genoms, is bisexual. A triploid with two maternal genoms and one paternal genom is also bisexual, but with the female tendency more strongly marked than in the bisexual diploid. An example among hepatics is the diploid bisexual clone of a normally dioecious *Pellia* studied by Showalter.

A somewhat different case is presented by *Sphaerocarpos*. In the capsules of those races whose spores are

regularly adherent in tetrads, spore dyads occasionally appear. Lorbeer found that the spores of such dyads give rise to females. Cytological study showed these females to be diploid, each possessing an X and a Y chromosome—having, that is, the chromosome complement characteristic of the sporophyte. I am able to corroborate Lorbeer's statements that the spores of dyads give rise to clones that are apparently female and that possess the diploid chromosome complement—although the possibility of an occasional deviation from exact diploidy is not yet excluded. Twelve such diploid clones have produced sporophytes in matings with haploid males—thus showing themselves functionally female. However, in sections of two of the diploid gametophytes, a few organs have been found similar to the intersexual structures described by several workers in hermaphroditic mosses. No such organs have been found in haploid plants of Sphaerocarpos. It appears probable, then, that the diploid clones are really bisexual, as are corresponding diploids in the other cases mentioned—although in Sphaerocarpos the female tendency almost completely dominates the male. Thus the results with Sphaerocarpos seem to agree with those derived from other dioecious bryophytes in showing that only one set of sex possibilities is represented in each haploid chromosome complement, and that evidence of bisexuality appears when both maternal and paternal complements are present.

Correns has applied to the analysis of the sexual conditions of bryophytes the same formula that he developed for angiosperms. He assumes the presence in both hermaphroditic and dioecious bryophytes of the genes (or gene complexes) A and G (the sex-potency factors), and in dioecious species of the additional genes α and γ (the sex-tendency factors). The presence of A and G in a dioecious species implies that each plant of the species possesses both male and female potentialities. But to this conclusion, inevitable for dioecious angiosperms,

dioecious bryophytes thus far afford no support. On the contrary, all the known facts indicate that a haploid male plant of such a species is and can be only male, and that a haploid female plant is and can be only female.

It is unnecessary, therefore, to assume that a dioecious bryophyte possesses distinct sex potencies and sex tendencies. Apart from sex-influencing factors, only one set is needed to explain the sexual conditions. This set might be likened to both the sex-potency and the sex-tendency factors of angiosperms. Perhaps, since homology is out of the question, and since genetic sex-determination here is so much more effective than in angiosperms, it may be allowable to speak of sex-*determining* factors.

In dioecious bryophytes with recognizable X and Y chromosomes, the sex-determining factors can hardly be thought of otherwise than as borne on these allosomes. Sex behaves in inheritance in a unitary fashion; it must be considered, then, as determined by a pair of factors or of closely linked factor-complexes. It would seem that the female determiner must be borne on the X chromosome. The male determiner being a positive thing, since its presence with the female determiner (in diploids) induces bisexuality, it is naturally thought of as borne on the Y. A hypothesis could be devised according to which a male-determiner is borne on an autosome, and its tendency, much as in Bridges' theory, is overborne by that of a female-determiner on the X. But such a theory is needlessly complicated for the facts in the bryophytes as now known, and, indeed, would not fit those facts so well as the simpler formula here proposed.

The formula is easily applied likewise to hermaphroditic species. In these, each plant has a male-determining and a female-determining factor, the two being borne on the same or on different chromosomes. If on the same chromosome, it can be imagined that in the sporophyte, where that chromosome meets its homologue, by loss or by interchange one chromosome of the pair loses

its female-, the other its male-determiner. Thereafter, half the gametophytic offspring would be male, the other half female—a dioecious race thus arising.

Although the evidence from varied sources indicates that hermaphroditism is the more primitive and dioecism a derived condition—its derivation in bryophytes possibly to be accounted for in the way just suggested—the probability is strong that hermaphroditic have in turn originated secondarily from dioecious species. Essentially this has happened repeatedly under observation whenever, by experimental means or otherwise, a diploid plant was produced which possessed the two sex-determiners. Heitz's observation that hepatics with 8 or 9 chromosomes are predominantly dioecious, while those with 16, 18 or other multiples of the basic number are predominantly hermaphroditic, suggests that hermaphroditic species have from time to time thus originated in nature.

The scheme suggested for bryophytes, then, involves a single female-determining and a single male-determining factor. Both are present in the chromosome complement of a haploid hermaphroditic species. In a dioecious species, each plant has only the female-determiner, borne on an X chromosome, or the male-determiner, borne on a Y chromosome. Diploid hermaphroditic species possesses both the X with a female determiner and the Y with a male determiner. It is judicious to anticipate the suggestion that discoveries may be announced to-morrow which will render this simple analysis inadequate. It fits the facts as they are known to-day.

PHYSIOLOGY OF EMBRYONIC SEX DIFFERENTIATION[1]

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LAST summer I heard one of our most prominent geneticists sum up past and future trends in developmental research with a rather striking parable. The geneticists have been searching Olympus, he said, for the gods which the embryologists were disrespecting on earth; what both ought to do, however, is to trace the hands of God in the works of nature. I confess that it sounded quite agreeably when genetics was leveled up to theology. Some of us will remember the time, only a few years past, when efforts to approach sex determination from a physiological as well as from the genetical point of view met with derision because they seemed to undervalue one of the most triumphant of recent biological discoveries and to exhibit the lack of a clean-cut conviction. That was when one X was equal to male and two X were equal to female. Though time has kept changing, McClung, first to point at the apparent correlation between odd chromosomes and sex determination (1902) was first again to propose a physiological interpretation based on the cytological peculiarities of the X-chromosome in spermatogenesis (1918). Taking up his suggestion, the following facts may be brought to light.

In the spermatogenesis of many grasshoppers, especially in orthopterans, the single X displays some odd features, such as heteropycnosis in interkinetic stages and lagging during mitosis. None of these peculiarities is exhibited in ovogenesis, which seems to suggest that the X is physiologically inactive in spermatogenesis.

This assumption agrees well with the result of genetical analysis, showing X as the carrier of the female determining gene.   It seems significant again that even in spermatogenesis the X behaves normally up to the stage of the second spermatogonia.   Before this, male and female germ cells are morphologically of the same type, and in cases of sex reversal it has been found that up to that stage they are capable of developing either into eggs or into sperms.   The peculiarities of the X therefore start at the time of the final sexualization of the individual cell.   At this point now arises the question whether they are the cause or merely a consequence of this sexualization.   The alternative, in my belief, has to be answered in the second sense.

We shall not enter into a discussion of the fact that the sex chromosomes behave normally in both sexes of the amphibians and of other animal groups—as shown by the cytological studies of Stohler and Makino on toads and my own on the frog.   We will only briefly consider the illuminating case of the stone fly, *Perla marginata,* where in the male a rudimentary ovary is found attached to the testes.   Junker has made a cytological investigation of these rudimentary hermaphrodites and reports that the haploid X chromosomes show heteropycnosis in the spermatocytes, while in ovocytes they become attenuated like the diploid autosomes.   This striking difference in the behavior of the X chromosome in ovo- and spermatogenesis seems to indicate that its odd features are a consequence only and not the cause of sex differentiation.   We admit that observational facts alone will never furnish binding proof of causal connections.   None the less, the presented case is fit to demonstrate that the sex chromosomes give not a solution to the problem of sex determination but rather represent one of its most intricate implications.   Moreover, the fact that male and female differentiation proceeds here on an identical chromosomal basis proves that the sex genes are no absolute rulers.   Cognizance of their flexibility will en-

courage the experimentalist.   The only way to get an insight into the mechanisms of nature is by imitation in experimental settings.   A study of the mechanism of embryonic sex differentiation has, therefore, to be based on experimental sex determination.

Unfortunately many workers in this field have had no further aim than to demonstrate the feasibility of artificial sex determination or of sex reversal.   The arrangement of their experiments is not so planned as to provide much insight into the physiological principles involved.   This is especially true for most of the plant work.   Schaffner, Yampolsky and others have been quite successful in upsetting Mendelian sex ratios in diverse dioecious plants.   One may generalize that they obtain their results by modifying, directly or indirectly, the nutritional conditions.   Many, though not all, conform to Goebel's rule that the male sex organs are formed under nutritional conditions, which are insufficient for the production of female ones.   This covers also the interesting observations of Cobb-Steiner and Caullery on some parasitic nematodes which are female in single infestations but male if living in large numbers within the same host.   However, general starvation has proved unyielding as a means of male production—proving that specific trophic factors and not the total food quantum are concerned.   Joyet-Lavergne in his physico-chemical theory of sex tries to account for this with his ''second law of sexualization.''   He contends that differences in nature and amount of lipoid and fat reserves constitute a fundamental and primary character of sex.

Female cells are accumulating reserves of fats.   The French author is somewhat contradictory about the question whether or not some of these lipoids are exclusively found in the female.   It is evident that the so-called law largely describes only the well-known facts of gametic sex dimorphism.   We are interested, therefore, to see how it stands the proof at critical points.   How does it meet, for instance, the facts of relative sexuality of lower

organisms? Max Hartmann and others were able to show that in isogametic thallophytes so called "weak males" and "weak females" are capable of functioning either as males or as females, according to the type of gametes with which they happen to come in contact. Evidently this relative sexuality excludes the assumption of specific lipoids, essential for the female sex.

It now remains to test the theory of characteristic proportions. Let us put the case of the Drosophila fly on trial. We can take for granted that the ovocytes have a higher fat content than the spermatocytes. Since the two types of sperms produce their well-known sex-determining effects the theory compels us to assume also that the X sperm has a relatively higher fat content than the Y sperm. However, we know through the non-disjunction work of Bridges that this X sperm determines a male zygote if it happens to fuse with an exceptional egg without X. Any general theory based on sex-determining cytoplasmic inclusions will be defeated at this point by the clear-cut facts established by chromosome research.

We can consider only briefly the metabolic theory, which is the objective of Joyet-Lavergne's "first law" and is best known through the extensive work on pigeons by Riddle. It says that the oxidation-reduction potential in a given species shows a lower value in the female than in the male. This again is the proper summary expression of a vast descriptive material as reported by Benedict, Dubois and others for man, Riddle for pigeons, Gayda for the toad, Hyman for Hydra, and so on. But experimental evidence of a causal relationship between metabolic level and sex differentiation is entirely wanting. In fact some experiments much rather point to the opposite. So the famous case of the Echiurid worm Bonellia. Since Spengel, we know that larvae settling down on the proboscis of some female develop into dwarf males. Baltzer was able to prepare extracts of female tissues which caused the same masculinization.

Recently Herbst succeeded in inducing an identical reaction simply by increasing or decreasing the hydrogen-ion concentration. He interprets this result as a consequence of a decreased respiration rate following any deviation from the optimal pH of normal sea-water.

My own temperature experiments with frogs have often been claimed to sustain the metabolic theory. Under the condition of a maximally raised temperature the ovaries of larval females transform into testes. If, however, an accelerated metabolism in itself were instrumental in male determination, we would have to expect some effect in this direction as soon as temperatures rise even slightly above normal. In fact, sex reversal does not start until we reach the point where the increase of temperature does not further accelerate the total metabolism, but gives it a distinctly catabolic character. Similar considerations apply to the male-determining effect of uterine overripeness of frog eggs. It would rather appear that an insufficiently low or an abnormal metabolism is somehow related to the masculinizing effect.

Differences in osmotic pressures, electrical potentials and hydrogen-ion concentrations have further been suggested as primary factors of sexualization. All these schemes suffer the same mistake as the metabolic theory, that is, to deny sex its own fundamental character. Sex certainly involves metabolic processes which sometimes may find a more exact expression in the readings of respiration manometers or potentiometers than in the delicate morphological changes which they entrain. However, we can not see that the more specific and basic properties of sex could possibly be expressed in terms of metabolism.

In a general theory of sex one would have to include the results of genetics and cytology as well as those of experimental embryology. The amphibians lend themselves favorably for research in problems of sex just because they can successfully be studied from either of these three points of view. The genetic and chromo-

somal analysis has disclosed that two genes are involved in the hereditary transmission; namely, a male factor which is localized in the autosome group and a female factor which lies in the sex chromosomes. The genetical sex formula resembles therefore that of Drosophila, save for the presence of a female factor (f) in the Y chromosome. The discovery in 1914 of this quantitative allelomorph of (F) was the first evidence of a gene localized in a Y-chromosome. In short, the genetic constitution is always *bisexual,* and a chromosome mechanism distributes the female genes in such a way that one half of the zygotes receives a relatively higher quantity than the other. The two classes are conventionally distinguished as genetical females and genetical males. Though, let us for once consider them more critically.

There are some local races of frogs in which the so-called males have ovaries all through their early development. They transform into testes only during the second half of the first year. In the same localities old females very often transform into males. We see here that the sex is not only a function of the relative gene quantity but also of age. Again, if we consider other local races which under average conditions always show the expected 1:1 ratio of males and females, we find that the "genetical males" develop ovaries at low temperatures while the "genetical females" transform into actual males at high temperatures. Therefore, sex appears this time as a function of genetic balance and temperature. For the interpretation of this double determination it is essential that breeding experiments with sex-reversed females show the original genic basis unaltered. Consequently, the interference of such factors as age or temperature must enter the play somewhere along the course of events that run between the genes and their final phenotypic manifestation.

I thought that this might give us a means to find out something about the working ways of the genes. While studying the effect of low temperature on the process of

sex differentiation in genetical males, I soon realized that the development of the medullar cords of the gonads was much more retarded than that of the cortex. At the same time ovocytes were beginning to form in the cortex, as soon as it reached a certain size, regardless of the genetical sex of the animal. Later, when the medulla had attained a larger size, spermatogonia were formed. Therefore, germ-cells of identical constitution differentiate in the female or the male sense according to their relation to cortex or medulla. Observations of this type led to the conclusion that cortex and medulla play a rôle of inductors, respectively. A nature experiment discloses a further interesting characteristic of these inductors. The anterior part of the sex glands of the toads consists of cortex only. In accordance with the theory just submitted it always differentiates into an ovarial lobe, in the male as well as in the female. Of special interest, however, is the fact that during the period of early sex differentiation the anterior lobe develops much faster than the posterior part of the gonad. The delay in this region where the functional ovaries and testes form, is clearly caused by a struggle for dominance between cortex and medulla. We realize, then, that each inductor is not only stimulating the differentiation of one sex, but it also tends to suppress its opposite. Cortex and medulla form an antagonistic pair of inductors. Their play is possibly best uncovered by the high temperature experiment. Young females transferred from normal to high temperature suffer degeneration of the ovarial cortex. Soon after, the medullar cords start a compensatory hypertrophy which ends with a perfect transformation of the ovary into a testis.

Sex determination offers now the following aspect. Female and male genes control the activation of cortex and medulla, that is, the female and male inductor systems. Dominance is not decided by gene quantities directly, but is the outcome of the competitive efforts of the antagonistic inductors to gain control. An asym-

metrical quantitative distribution of the female genes through the sex chromosome mechanism may regulate the final sex determination under certain conditions. In general, however, environmental factors, favoring or disturbing selectively the development of the inductor systems, help to determine the course of the embryonic differentiation.

From the foothold thus gained, it is possible to explore further into the field of developmental physiology. We have especially been interested in the process of induction. Burns was first to apply Born's technique of amphibian parabiosis in sex research. Humphrey and myself, with a number of my students, have contributed in accumulating data that give now a well-rounded picture of some phases of the inductive process.

Embryos of frogs or of salamanders were grafted together so as to form twin pairs, long before the stage of sexual differentiation. According to the principles of chance combination, it is legitimate to expect that half of the pairs consist of a genetical male and a genetical female. In the case of salamanders and newts the male twin generally develops normal testes, while the ovaries of the female, after some initial differentiation, are reduced in size and finally become nearly or completely sterile. In exceptional cases, if the ovaries of the female get a sufficiently early start, the reaction appears reversed. We speak of a "free-martin reaction," because the effect is much the same as described by Lillie for chorionic twins in cattle. The same interpretation certainly will serve both the urodele and the cattle case. Cortex and medulla effect their inductions by releasing specific hormones, each interfering with the normal development of the other. If the two hormones appear at about the same time, the male one soon puts the female inductor out of action. Female dominance is only established if the ovaries attain a considerable size before the testes become differentiated at all.

The same experiment gives a somewhat different result with frogs. Here, ovaries and testes both develop unimpaired, if they are located far enough apart. In case they come closer together, those parts of the ovaries that lie nearest to the testes suffer first the arrest of development. Altogether, we notice here that the inductive effect spreads with a falling gradient, a mode which resembled more the type of embryonic induction which became known through the work of the Spemann school. As in the urodele twins, induction goes out from both ovaries and testes, male induction finally taking the lead in all cases.

The same experiment a third time repeated with toads does not reveal any signs of inductive interference. Induction apparently is confined within the sex glands. One is tempted to speak of an ascidian type, since Conklin established the lack of inductive transmission in Styela and Ciona.

So far I have been speaking of inhibitory induction only, since this is best worked out now. Evidence of stimulative induction is accumulating, however, which gives proof of the same three types of transmission.

Induction, generally speaking, seems to be effected by substances which are specific with respect to origin, chemical nature and substratum on which they act. In the three types just described these substances are so much alike regarding their origin and their physiological properties that we must assume a close relationship. In other words, we feel justified now and even compelled to consider formative substances and hormones as belonging in one class. It is in agreement with this interpretation when Conklin finds that the formative substances of the ascidian blastomeres are not identical with mitochondria nor with any other visible cell inclusion. Successful experiments of Spemann and his collaborators to obtain morphogenetic induction with non-living débris and with extracts prepared from amphibian organizers

add support to the notion of a hormonic, *i.e.*, a relatively simple chemical nature of the inductive substances.

We have to pay tribute to the genius of Jacques Loeb, who, years ago, had already conceived this solution in general terms. We remember also that Goldschmidt gave Loeb's idea a central place in his ''physiological theory of inheritance.'' Nor shall we forget, however, that we have barely started now to provide this theory with a substantial basis of experimental facts.

The physiology of embryonic differentiation has long been one of the cardinal problems in biology. Up to recent times development has either been considered as a creative process or else as a mere growth of preformed minute germs into visible proportions. The aspect has fundamentally changed when Mendelian genetics revealed the existence of a complicated implicit structure of the organism which is by far more durable and less modifiable than its ever-changing and in generations pulsating explicit form. How are genetical constitution and phenotypical manifestation linked together? In the approach of this great problem it appears that embryonic sex differentiation offers unique research possibilities. It is the field in which genetics and embryology have been most successful, so far, in the much-needed and much-endeavored cooperation.

SOME GENETIC ASPECTS OF SEX[1]

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I. SEXUALITY

FROM the genetic point of view it is advantageous to begin by considering sex in the broader sense of sexuality. It is not generally realized that genetics has finally solved the age-old problem of the reason for the existence (*i.e.*, the function) of sexuality and sex, and that only geneticists can properly answer the question, "Is sex necessary?" There is no basic biological reason why reproduction, variation and evolution can not go on indefinitely without sexuality or sex; therefore, sex is not, in an absolute sense, a necessity, it is a "luxury." It is, however, highly desirable and useful, and so it becomes necessary in a relativistic sense, when our competitor-species also are endowed with sex, for sexless beings, although often at a temporary advantage, can not keep up the pace set by sexual beings in the evolutionary race and, when readjustments are called for, they must eventually lose out. Thus sexual beings form most of the central and the continuing portions of the evolutionary tree from which ever and again new sexless end-twigs sprout off.

Whatever the secondary needs of present-day somatoplasm may be, there is no fundamental protoplasmic need for rejuvenation of the germ plasm through sexual union, no reason to believe that "protoplasmic stimulation" is *per se* produced by mingling of unlike germ plasms, nor any evidence that variation of the hereditary particles is induced by "panmixia." A more reasonable claim might be made out for the new genetic concept of "heterosis" as furnishing the function of sexuality and

[1] Paper read at the symposium on "The Biology of Sex" before the American Society of Naturalists, New Orleans, December 31, 1931.

sex. By heterosis we mean the increased vigor of hybrids, as compared with pure breeds, which is caused by the preponderant dominance of the genes favoring survival and growth furnished by both parents. But a more searching study of this matter shows that, in the main, heterosis affords only a compensatory advantage, in that it makes up for deficiencies that sexual reproduction is itself mostly to blame for. Heterosis arises only when cross breeding is wider than it has been on the average in previous generations. But if this wider cross-breeding is kept up, deleterious recessive genes will accumulate until a new equilibrium is reached, at which stage there is a sufficient abundance of such genes to cause even these more "mixed-blooded" individuals to exhibit as many recessive defects as did their "purer blooded" ancestors. *Vice versa,* if we increase the intensity of inbreeding, the more rigorous selection ensuing will eventually lead to the inbred line being purged till it has as great vigor as its more cross-bred ancestors. The closer the inbreeding, the less does sexual reproduction depart, in its genetic effects, from asexual reproduction, and we may conclude that at the limiting state, that of asexual reproduction, there would not (after the attainment of a state of equilibrium) be less vigor than in sexual organisms. The attainment of equilibrium in regard to the number of harmful mutant genes present may, however, require a very considerable time, and in the meantime sexual reproduction would be of advantage through its induction of heterosis. Heterosis may therefore have been of immediate value, in the first origination of sexuality, and so it may explain how sexuality happened to become established in the beginning, as Altenburg has suggested in an as yet unpublished work. But heterosis can not explain the major function of sexuality and why it has persisted in the long run, and acquired such complicated accessories.

Among the primary and accessory features of sexuality there must be considered not only the differentiation of

male from female germ cells, the differentiation of male from female sex organs, the separation of the sexes, with its associated mechanism of sex determination, and the differentiation of secondary sexual and ''sex-limited'' characters in general, but also the mechanism of Mendelian heredity itself, involving segregation of homologous chromosomes, independent assortment of non-homologous chromosomes and crossing over. Without sexual reproduction, the latter mechanisms are not called for, and would not continue to operate. Which, however, among the attributes mentioned, occupy a more primary and which a more secondary status? It is clear that not only is sexual reproduction necessary for the operation of segregation and recombination of chromosomes and chromosome parts, but, conversely, the latter are necessary in order that sexual reproduction may have any permanent value, while all the other characteristics of sexuality, though enhancing, are dispensable. Of the two major features, segregation and recombination,[2] only recombination is in itself of evolutionary value, but it can not take place without segregation and so we must suppose the two to have sprung into existence at nearly the same time. Mendelian heredity must therefore have arisen almost full fledged, when sexuality arose. This complicated step, which probably required a peculiar concatenation of accidents, along with selection, seems to have been taken in the green algae, and from them to have been inherited by animals and higher plants alike.

The essence of sexuality, then, is Mendelian recombination. Not increased variation in the sense of more change in the hereditary units or genes, now that we know there are these units, but the making and the testing out of all sorts of combinations among these gene mutations which would arise and become evident any way. Sexual-

[2] It is not possible at present to decide definitely whether recombination of whole chromosomes or crossing over was first evolved; either would have been sufficient to give value to sexuality. But it seems more probable that crossing over was a later development.

ity, through recombination, is a means for making the fullest use of the possibilities of gene mutations; thus it is itself an accessory process, accessory to the primary process of gene mutation.

There are two ways in which recombination of gene mutations is valuable. One, by far the lesser way, is the providing of an opportunity for continual shifting and readjustment of the relative abundance of different types as external conditions vary back and forth, and here and now one, there and then another combination becomes more advantageous for the maintenance of the species. In this process heterozygosity is an asset, and the disadvantageous combinations continually produced are an insurance against the day when some of them will be needed.

The other, the major value of recombination, is the production, among many misfits, of some combinations that are of permanent advantage to the species and that eventually become fully established in it as a part of its normal constitution. Without sexual reproduction, the various favorable mutations that occur must simply compete with each other, and either divide the field among themselves or crowd each other out till but the best adapted for the given conditions remains. In asexual organisms, before the descendants can acquire a combination of beneficial mutations, these must first have occurred in succession, within the same lines of descent. In sexual organisms, however, most of the beneficial mutations that occur simultaneously, or in different original lines of descent, can increase largely independently of one another and diffuse *through* one another, as it were (see Diagram 1). (Our diagram does not accurately represent this spread of genes through one another; it would hold only if the individuals and genes were fixed in geographical position and unable to disseminate freely amongst one another. If their positions were completely random, we should need a new dimension, at right angles to the previous ones, to represent the diffusion of each

new mutation. The actual situation lies somewhere between these two extreme alternatives.)

EVOLUTIONARY SPREAD OF ADVANTAGEOUS MUTATIONS

IN ASEXUAL REPRODUCTION; IN SEXUAL REPRODUCTION
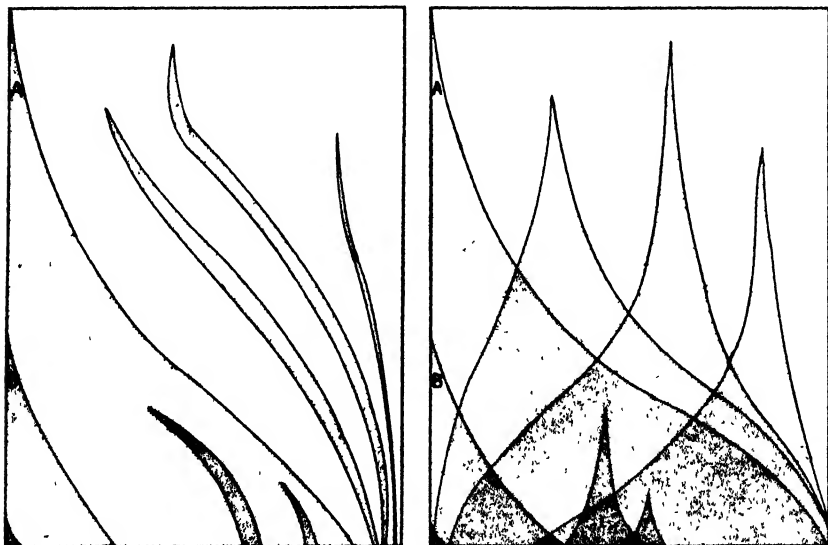
DIAGRAM 1. Showing the method of spreading of advantageous mutations in asexual and sexual organisms, respectively. Time is here the vertical dimension, progressing downwards. In the horizontal dimension a given population, stationary in total numbers, is represented. Sections of the population bearing advantageous mutant genes are darkened, proportionally to the number of such genes. In asexual organisms these genes compete and hinder one another's spread; in sexual organisms they spread through one another. See, however, qualifications in text (p. 121), explaining limitations of a diagram in only two dimensions. The diagram is simplified in a number of other ways as well. For example, all mutants represented are shown as spreading at nearly the same rate, if they do spread, and this rate is shown as about the same regardless of the extent to which they have entered into combination with one another.

Now it can easily be shown that the ratio which (on the average) the number of individuals in the most favored line of descent, counting from the time of occurrence of one favorable mutation (A) to the time of occurrence, within the same line, of the next favorable mutation (B),

bears to the number of individuals in the population as a whole in the same period (in Diagram 1, the ratio of the left-hand mutant shaded area A to the total area, in the region between two horizontal lines drawn through the points of origination of mutant shaded areas A and B) would represent roughly the speed of evolution in an asexual as compared with a sexual organism,[3] provided a correction, making the situation still more favorable to the sexual organism, is made here, namely, multiplication of this ratio by a factor representing the greater speed of increase of the favorable mutations in the sexual than in the asexual organisms, due to the fact that in the former the different favored mutations do not have nearly so much tendency to interfere with one another's increase. When such calculations are made, using any reasonable-seeming premises for mutation rate, selection and population size, within very wide limits, it is found that the advantage of sexual over asexual organisms in the evolutionary race is enormous.

In these calculations, and in the diagram, the assumption has been made, for the sake of simplicity, that the advantage of a mutation is the same regardless of the combination in which it occurs. However, the value of a combination of mutations will sometimes be far greater than the mere sum, or even the product, of the values of each mutant condition taken separately. Therefore, as Wright has recently pointed out, it is sometimes possible, by means of recombination occurring before selection, to get valuable combination-types which would not have come into existence at all, or only with far greater difficulty, if the "complementary" mutations composing them had had to occur and then to become selected in succession, as must happen in asexual reproduction.

[3] For while, in the given time, only one new advantageous mutation (B) became available in the favored line (A) of the asexual organism (the mutation rate being such as to give one in this number of individuals), in the sexual organism as many new advantageous mutations would become available, for combination with A, as the area of A goes into the total area.

While it is true that only the findings of modern genetics could enable our conception of the function of sexuality to take on the definite form above outlined, and only they could furnish real proof of this conception, nevertheless it should be recognized that the core of the idea—the formation of new combinations of "determinants," having a selective value sometimes greater than the original combinations—was conjured up long ago by the genius of Weismann, who herein, as in a number of his other major contentions (non-inheritance of acquired characters, reduction division), to-day stands brilliantly confirmed.[4]

II. On the Origin of Sex and Sex Determination

The advantage of the division of labor between sperm and eggs has long been obvious to biologists. Perhaps it is also needless to point out that the further differentiation, leading to the existence of the two sexes in separate individuals, is of advantage in the same way as any other division of labor in which more individuals than one are mutually involved, in this case rendering the performance of the respective tasks of finding a mate (or causing the male gametes to reach the female), and of giving the offspring a good start in life, respectively more efficient. But in cases where conditions are such that these functions in the same individual would not greatly interfere with one another—as is often true in organisms that are slow-moving any way and that need not be otherwise even for mate-finding—the efficiency may not be increased enough by dioeciousness to compensate for the effect of the latter in halving the number of individuals giving each type of gamete and in reducing the proportion of contacts which would be of service in fertilization, and so these organisms may have retained or developed hermaphroditism. This relation too has been pointed out by Altenburg in the work above referred to. His con-

[4] I am indebted to Professor S. J. Holmes for having redirected my attention to this important historical fact, subsequently to my address at New Orleans.

tribution concerning the relation of ''reproductive load'' to hermaphroditism is especially valuable in this connection, but we do not wish to anticipate it here. On the other hand, in dioecious species, it is not so evident why it should be most advantageous for them to have almost exactly equal numbers of the two sexes, as is usually the case, but perhaps this proportion exists simply because it is the easiest to produce, genetically.

If, as seems likely, hermaphroditism was the more primitive condition, dioeciousness may sometimes have arisen, as recently obtained artificially in corn, by means of two separate mutations which caused male-sterility and female-sterility, respectively. These mutations may in some cases have been linked (lying in homologous chromosomes), but such an arrangement would not be in the direction of the more prevalent mechanisms of sex-determination, in which the Y or W chromosome is relatively unimportant. More likely the second mutation (say, that causing female-sterility) was of a ''sex-limited'' type, such that its effect could be produced only when the effect of the first mutant gene in question (say, that causing male-sterility) was not being produced. Thus the second mutant gene would tend to become homozygous throughout the population and yet the effects of the two mutant genes would remain alternative. The first mutant would come to be heterozygous in half of the individuals and homozygous in the rest; it would have to be regarded as the ''sex-determiner'' proper. But it would seem a long way even from this kind of dioeciousness to one in which the sexes are automatically almost completely alternative, as in some animals that have been studied, where the development of a set of characteristics of one sex, in any given part of the body at any given stage, whether owing mainly to genetic or environic influences, necessarily goes along with a corresponding inhibition of a whole set of characteristics of the other sex. Many adaptive ''modifying'' mutations would have had to become established by selection, to

make the process controlled by the sex-determiner so effective, in causing the development of the characters of each sex to occur to the exclusion of those of the other sex.

As shown first by the work of Goldschmidt on the gipsy moth, the same genetic configuration may lead to either full maleness or full femaleness, for a given part at a given stage, independently of other stages, the result at that point depending on the strength of a particular developmental influence or influences, and in special cases the strength of the influence may change gradually during development so as to cross the critical level separating the two sexes. As the crossing of this level seems to involve a cleanly alternative alteration in nearly all sexual characteristics at once, that are scheduled to undergo development at the stage in question, it seems likely that the determining influence in question is a single one, *i.e.*, that there is normally a single "focal" process of development, or a single kind of developmental material, that is sex-deciding. That the same is probably true in Drosophila may be deduced from the recent work on intersexes by Dobzhansky and Bridges, extending Goldschmidt's principles to this organism. Further evidence of the largely unitary character of this sex-deciding process is to be found in the fact that the mechanism of sex determination has time and again changed, and that when the change occurred it could scarcely have been by a series of small steps, as would have had to be the case if many independent processes had been involved.

It is reasonable to suppose that the present scheme of sex-determination in Drosophila, for instance, arose as the result of a mutation which affected the strength (*i.e.*, the intensity or concentration) of the process or substance in question. This mutation need not have represented the first origination of the dioecious condition or of the sex-deciding process in question. The pair of allelomorphs thereby established may merely have superseded another allelomorphic pair, or another set of alternative conditions, which previously had had the sex-

deciding rôle, just as must have happened when what I have designated (in "The Mechanism of Mendelian Heredity," 1915, p. 83) as the "WZ" method of sex determination of butterflies and of birds superseded the previous XY type more generally characteristic of insects and of vertebrates, respectively. We are not compelled to conclude that the new gene or pair of genes that had this deciding effect was the one that synthesized the "focal" substance, or that chiefly carried on the "focal" process in question. No doubt, as in the production of other characters, this substance or process too depended, and depends, on many genes, some more and some less important, some helping to determine its nature, others only influencing its "strength," or allowing it to exist (see Diagram 2). In the same way, whether or not a pistol shall be fired may depend upon various details of the nature of the mechanism, upon the powder or merely upon the pulling of the trigger. To which of these possible categories the newly deciding gene belonged can not now be ascertained. But this single mutation must by itself have been enough to allow the gene in question to become fully sex-determining, *i.e.*, to decide between a fully functional male and functional female, otherwise the mutant would not have been able to survive.

Altenburg (*op. cit.*) has pointed out that there is at first sight an apparent genetic difficulty encountered in accounting for the origination of the above mutation, inasmuch as the work on non-disjunction shows that the X is far more important than is the Y in sex determination and that therefore the male represents, in effect, a haploid condition of sex-deciding genes present in diploid in the female. We may account for such a situation in one of two ways. On one interpretation we take as our point of departure the genotype of a male or hermophroditic individual, not containing the present sex-determining gene or genes which I have designated as "S" ("Mech. Mend. Hered.," 1915, p. 78) which now exist in the X chromosome, but homozygous for an earlier allelo-

morph, which we may call "s," and which we must suppose to be indifferent in its effect on sex. By means of a "positive" mutation (*i.e.,* one different in its character from a loss, and similar to Hairy wing, Blond and Bar in Drosophila—see Muller, League and Offermann, 1931, *Anat. Rec.,* 5: 110), the indifferent gene "s" would have had to become changed to the sex-deciding gene "S," which had to be present in double dose (SS) before its effect of suppressing maleness (while allowing development of femaleness) could be produced. The indifferent allelomorph "s" in the "Y chromosome" remained equivalent to an "absence," so far as its effect on the sex characters was concerned, just as the normal allelomorphs of Hairy wing, Blond and Bar are equivalent in their effects on these characters to absences.

On the other interpretation, we start from the homozygous "SS" individual (female or hermaphrodite) as a base. We must now suppose that a mutation of S occurs which is similar in its effect to a loss, producing an indifferent gene, or an absence, "s." This lesser gene, or loss, s, may be said to dominate over S, in the sense that the one dose of S, in the combination Ss, has a different effect than the two doses, SS, this difference being sex-deciding. The peculiar feature of this situation is not that one dose has a different effect from two, but that the sex-determining mechanism should already have been so prepared in advance, as it were, that a mere loss was all that was necessary to produce the whole change-over. This becomes understandable, however, if we postulate in this case that the "focal" sex-deciding substance or process had been previously evolved, through a series of changes in other genes but that a different gene or other agent had hitherto been determining whether or not it should occur. Once having gone through this evolution, its strength would be influenceable by changes in various contributing factors. Of these, the sex gene "S" here in question was merely one.

In view of these considerations, we should also expect that in the future changes in still other genes might be able to exercise the deciding influence and so take over the function of sex determination, in which case, in all probability, other chromosomes would become sex-determining. Moreover, there is nothing in the mechanism projected which would, *a priori,* make it impossible for quite different kinds of influences, such as special external conditions, or the haploid-diploid difference, or the stimulus of fertilization, to take over the function of the pulling of the trigger of the already evolved sex-determining process. But it would be to the advantage of the organism if the sex-determining process were not easily influenced by ordinary environic differences, for then the sex ratio would be too easily upset, and so we should expect the process to have developed safeguards against being readily changed in such ways to the degree necessary to cause the change-over.

III. Singular or Plural ''Sex Genes''?

The fact that originally there must have been just one gene that played the critical rôle in the sex-deciding process does not mean that, as time went on, and the X-chromosome became, by mutation pressure, increasingly differentiated from the Y, other genes did not finally come to be contained in the X which also, through the difference in the effect of their dosage in the one-X and two-X conditions, affected the process in question. Some of these may work in the direction of strengthening, others of weakening this process (when their dosage is increased), but the sum total of their activities, together with that of the original sex-determiner, must be about the same as the latter originally was by itself. So there is as much likelihood that the X-chromosome contains ''minus'' sex-genes—those working in the male direction—as ''plus'' ones, and if such exist there must be an intra-chromosomal balance between the two. Recent work of the author, League and Offermann (1931,

op. cit.; see also Muller, 1930, in *J. Gen.*, 23) indicates that in the X, owing no doubt to its peculiar history, the intra-chromosomal "genic balance" is peculiarly delicate and intricate, in regard to the determination of various traits, and sex should be no exception to this.

As I pointed out in 1928 (Muller and Altenburg, *Anat. Rec.*, 41: 100) breakage of the X-chromosome by x-rays will enable us to determine the approximate locus or loci of the present sex-deciding gene or genes in the X, along with the real map of other genes. Hence, if the sex genes are not too numerous, their number and their relative effects also can be determined in this way. My work on broken chromosomes had already showed at that time that not more than half of the X contained genes of consequence in this connection. Since then the work has progressed a good deal further, chiefly through genetic studies of Patterson on broken chromosomes in mosaic flies in which the normal half of the zygote allows the other half, having the broken chromosome, to live and have its sex ascertained. As I suggested in 1930 (Muller and Stone, *Anat. Rec.*, 47), the inviability of the individual having a large piece of the X broken or missing, which is our chief difficulty in such studies, should also be obviable, to some extent, by carrying out our studies on flies triploid for the autosomes, since here the genic disproportion would not be so great. Following this method, Dobzhansky and Schultz (*Proc. Nat. Acad.*, 1931) recently report finding that several parts of the X have some positive influence in sex determination. It is too early to make a digest of the whole matter, but we can at least say that some parts of the X of *Drosophila melanogaster,* including all genes to the right of forked, are practically without influence in sex-decision, other parts, in the extreme left end, are of little if any influence, and in the remainder the influences are at least unequal, and are being gradually traced down. For the further definite findings of importance along this line the reader may be referred to the very extensive work of Patterson

(abstracted, 1931, in *Anat. Rec.*, 51: 111), which he has summarized for us at the present meeting, in his address to the genetics sections.

In all the above discussion it has of course been taken for granted that it is not the absolute amount of the sex-deciding gene or genes which determine sex (still less the quantity of the X-chromosome material as a whole), but the amount relatively to that of other genes, with the products of which those of the sex-deciding genes react. There is no use detailing here the controversy into which my advocacy of this view brought me in the early days with other Drosophila workers, notably Sturtevant, who cited in opposition sex determination in Hymenoptera, which I maintained was controlled by a totally different type of trigger, but its final vindication came, as is well known, with Bridges' discovery of triploid females and intersexes in Drosophila and his more recent demonstration of haploid female tissue as well. You can not tell how sweet a cake is merely by knowing the amount of sugar used in making it, you must also know the amount of the other constituents and its total size: the same principle holds in sex determination. By the same token, too, we must conclude that the amounts of various specific autosomal genes are also important in sex-determination and, by means of breakage produced by radiation, we should eventually be able to trace down these genes also, and gain information concerning their rôles in the sex-deciding process.

We can not, in this short space, discuss or even pretend to outline the problems in the whole field of the genetics of sex, but it should not be forgotten that what I have called the "sex-deciding" process is but one process, and maybe a relatively small one, in the whole series of processes that interweave in the production of all the male and female primary and secondary sex characters and many so-called sex-limited characters (see Diagram 2). I have called it a "focal" process because, firstly, no doubt the reactions set up by various genes converge as

SEXUAL CHARACTERS
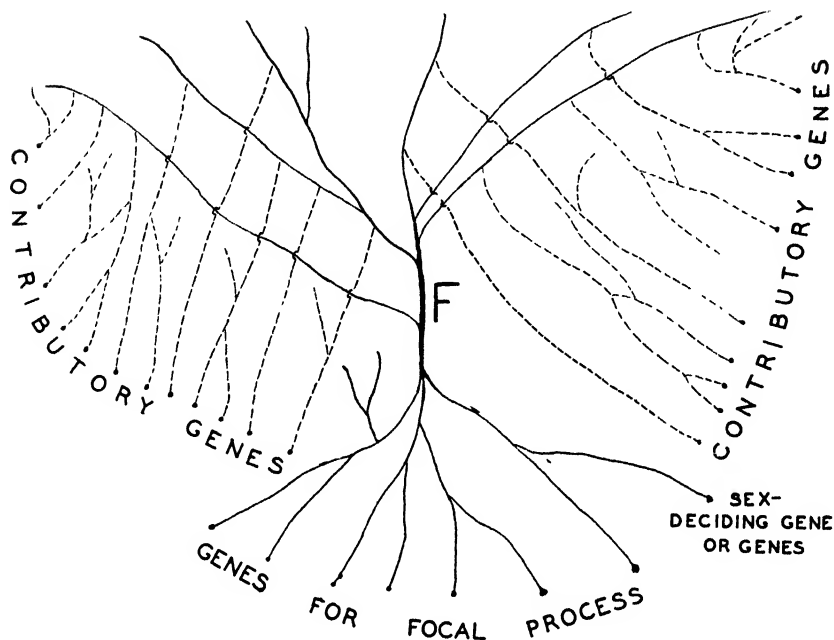
DIAGRAM 2. Schematic representation indicating the kinds of interrelationships existing between the final sex characters and the genes which determine them. Processes (physical and chemical, leading to morphogenetic effects) are indicated by lines, lines convergent from below upwards indicating interactions, lines divergent from below upwards indicating plural effects. The processes are represented as commencing with the genes below, and progressing upwards to the visible characters, above. Environic influences are not represented. Only one sex is shown. The "focal" or "sex-deciding" process, F, has the rôle not only of leading to the production of the characteristics of the one sex, but at the same time of inhibiting those of the other sex. This inhibition is not indicated here. Moreover, no distinction is made here between the direct production of a process and the "inhibition of an inhibition" of it, since the final effects are the same and a practical distinction can not usually be made. The diagram is admittedly vastly over-simplified, and is not intended to show the concrete relations of the processes, but only the kinds of relations existing among them.

to a focus, to make it what it is, to determine its nature and its intensity, and secondly, this process in its turn has multitudinous effects, lines of action diverging from it, as from the thither side of a focus, since the process

is necessary for the setting into action of all the various other processes which result in the production of all the different sex characteristics. But this multiplicity of effect also requires the cooperation of innumerable other genes, which, though not sex-deciding in the above sense, are certainly concerned with sex and are thus in a sense sex-genes. It may even be true that, to a slight extent, the majority of genes are influenced in the amount of their effects by the concentration of the sex-deciding genes, and are in this sense partially sex-genes, inasmuch as they tend to add to the sexual dimorphism.

IV. Rôle of the Y Chromosome

I have neglected the Y-chromosome in the above account, because the Y has neglected itself. According to the hypothesis of the origin of the Y which I published in 1914 (*J. Exp. Zool.*, 17: p. 326–328; see also *Gen.* 3, 1918, p. 479–484) the genes of the Y have gradually undergone inactivating and loss mutations, from the effects of which the organism has been largely protected, through the continual presence of an X having normal (or "hypermorphic") allelomorphs. In other words, the Y has paid the penalty always exacted by the protection of continual heterozygosis, and the consequent absence of natural selection. The largely inert Y may subsequently undergo changes in size and shape without detriment to the organism, and so tends to become visibly different from the X, luckily for cytologists and geneticists.[5] But it must

[5] Recent evidence (see Muller, League and Offermann, *op. cit.*) suggests that, although the individual "loss mutations" or "hypomorphic mutations" of the Y were not detrimental enough to prevent them from finally becoming established by mutation pressure throughout the population, nevertheless in the case of some of them, the ensuing one-dose condition of the genes of the X in the male may have exerted some sensible detrimental effect. If so, this effect was later compensated for by the selection of modifiers, which made the males with one dose about equal, in degree of expression of these X-chromosomal genes, to females with the two doses. If this interpretation is correct, loss of a whole section of the Y would have been detrimental, at a time before this piecemeal loss, accompanied by piecemeal compensation, had taken place. Alternatively, we may sup-

retain enough genes to allow it to act as the homologue of the X in segregation, if it is to persist at all, and, if any dominant genes exist or arise in it, which are advantageous to the sex in which Y occurs exclusively, they may be retained by natural selection. With regard to these male-helping genes we may observe the following: as it is a rule of evolution that characters at first merely an asset will, if they are retained long enough, finally become, through correlative evolutionary changes, a necessity, we find that at present the Y of Drosophila, though relatively unimportant in sex determination, nevertheless contains genes or gene-complexes, "k_1" and "k_2" found by Stern, 1927 (*Die Naturwiss.* Jahrg. 15) that are essential for the complete functioning of the male.

The Y, having become nearly inert, may also now serve as a source of inert chromatin that may become translocated into other positions, and even serve as an anchorage for the formation, by translocation, of new autosomes chipped off the old ones. At least, on the basis of the studies which have recently been carried on by Stone, Painter and myself, I have reached the conclusion that, by translocation, a large part of the Y of Drosophila has become engrafted on to the original X, so that only about half the length of the present X consists of original X-chromosome material, the rest consisting of inert material derived from the Y, material in the main unable to mutate, to undergo crossing over, or to function in morphogenesis (Muller and Painter, 1932, *Z. ind. Abst. u. Vererb.*, in press). Its function, if any, still remains a mystery, but the possibility of its existence in unsuspected situations must be taken into account in future cytological studies. Here we have one little example of the numerous ramifications of the secondary effects which sexual differentiation has led to.

pose that these "compensated" genes of the X are "neomorphs," functionally unlike any that ever existed in the Y. But even in that case, *duplications* of the Y or of whole sections of it could not have been innocuous until it had become nearly "empty."

V. Significance of Sex Studies

The genetic study of sex is important not merely because, according to Freud, sex is instinctively our major interest, a proposition which is at least disputable, even for *Homo sapiens biologensis*. It is important also because, as we have seen, it lies at the root of Mendelian heredity itself and is one of the major factors in evolution, even though it is not, in an absolute sense, necessary. Thirdly, it is important because it provides such admirable material for the study of gene interaction, of phaenogenetics, that is, of *Entwicklungsmechanik* from a genetic standpoint, together (fourthly) with the associated study of the evolutionary processes whereby these developmental complications arose. Sex and sex characters are peculiarly adapted for this purpose because, while they constitute a highly complicated mechanism developed through a long evolutionary sequence, nevertheless they can be dissected apart, practically down to their very root, by a combination of mutational, embryological and physiological means, without necessarily killing the organism (as witnessed by the results cited in the other papers in this symposium). This possibility arises from the fact that the vegetative functions can go on without reproduction and that the organism, through its possession of two sexes, must be already adapted to carry on either one without the other. Most other complicated systems—circulatory, digestive, excretory, etc.,—if much tampered with, soon result in lethal effects, and thus largely foil the knife of the genetic and morphogenetic investigator. Within the life system as a whole, then, we have here a contained system, the sex system, sufficiently independent so that it can be vivisected down to the point of extirpation without death ensuing, and which can therefore be used as an object of research to illustrate the general principles of gene interaction, morphogenesis, physiology and evolution pertaining to the life system as a whole.

APPENDIX   .

The reader may wonder why the letter "S" has been used in the preceding article to represent the sex-deciding gene or group of genes, rather than the letter "F," for femaleness, and why "M," for maleness, has been omitted completely. He may also think that some references should have been made to the development of these F- and M-containing formulae for sex, and to the controversy concerning to whom the credit for these conceptions should be given. In view of the prominence which has been given to this topic even in some of the more recent literature, the author ventures to present here a portion of a paper entitled "Erroneous Assumptions regarding Genes," which he wrote while at the Cornell Medical College in the winter of 1911–12, and which he did not have opportunity at that time to publish. It will be evident from this paper that even at that time the "FM" formulation was no new one, but that the grounds for its rejection were already extant. As a result of the ideas of the author advanced therein, and others added by him later, the representation "SS, SO" was used in the "Mechanism of Mendelian Heredity," 1915, 1923, and the criticism "What are Sex Factors," was presented in the same volume (see especially pp. 90–97 and p. 107 of the 1915 edition, or pp. 90–94 of the 1923 edition, which it may be of interest to reread in the present connection).

. . . We may in conclusion undertake a criticism of certain needless assumptions that are sometimes made regarding sex itself, for this may furnish an illustration of the applicability of the above line of thought (that the processes through which the genes accomplish their outward effects may be extremely complex and interrelated) in other directions. When we find that the male, for instance, is heterozygous for sex, the female homozygous, then, in the absence of any cytological evidence, there is only one justifiable formula by which the sexes can be expressed, namely $XX = ♀$, $XY = ♂$; or, if there is evidence from cytology that the X chromosome has no mate, the Y may be changed to O. All other formulae are unwarranted modifications of these two. Thus, Morgan, in an attempt to account for maleness in Drosophila (where the Y is absent and the scheme is $XX = ♀$, $XO = ♂$[6]),

[6] Just at that time, the Y was erroneously thought to be absent from Drosophila. Nevertheless, as it has since been found to be nearly "empty" genetically, this formulation will hold approximately anyway.

introduces an M into the formula and changes X to F. His formula for the ♀ is then FMFM, that for. the ♂ FMM, where F, femaleness, stands for X, and M, maleness, is not sex-linked. Both of these assumptions are unwarranted. It may well be that not only factors necessary for femaleness but also factors for maleness abide with the X chromosomes. This may be true not only of sex characters common to both male and female . . . but even of distinctively male characters, or generalized ''maleness'' itself, if that exist. . . . For, just as a gene in haploid amount need not produce an effect equal in quantity to that of the diploid amount . . . , so too its effect may differ also qualitatively, when haploid, from the diploid effect. It is not uncommon for the effect produced by an agent to vary in quality as the agent varies in quantity. Thus, to build upon an idea of G. H. Shull's, a certain amount of alkali added to an acid solution yellow with alizarin, may change the latter to an orange color, whereas twice as much may make it deep purple. Therefore it is quite possible that XO = male, XX = female, without any non-sex-limited M, and therefore too that X is not identical with a hypothetical F, femaleness.

It is not even necessary, however, to postulate here that a certain gene in haploid number produces an effect different from that in d ploid. We may instead assume that certain dominant factors for maleness exist ''in'' the X chromosome, but that there also exists in that chromosome a recessive inhibitor for this factor or factors (either a simple inhibitor or a complex of several genes that may have other side actions as well). We say recessive, meaning that the haploid quantity is ineffective. In this case the composition of the X chromosome, if represented according to the scheme of the preceding formula, would be MI; this too would represent the male, one I not being active, and M thus producing maleness. The female would be MIMI, the two I's effectually inhibiting maleness. In this case we could consider femaleness to be a non-sex-linked character or aggregate of characters, thus partially reversing Morgan's formula of FFMM = ♀, FMM = ♂ by putting MIMIFF = ♀ MIFF = ♂ ; we could, however, as well consider that ''femaleness'' (meaning all or a part of the factors necessary for femaleness) also was sex-linked, but recessive, like I, not dominant, like M. Then we would have MIF = ♂ ; MIF MIF = ♀. If we chose, we could simplify the formula by the omission of I, regarding F (or even M) as possessing its properties, especially since by the letters we are not strictly denoting single genes, but possibly collections of them. Then we would have MF = ♂ MFMF = ♀, M dominant to its absence, F recessive to its absence and inhibiting M. If likewise in the previous case (MIMIFF = ♀, MIFF = ♂) we considered I as being merely a property of M, we would have MMFF = ♀ ; MFF = ♂.

In all the above cases, we have for purposes of comparison used the scheme of representation of the formula under criticism; we do not favor this method of representation in general. For, as we pointed out in another connection, a single letter is used in Mendelian notation to represent a single gene, but, in cases where the factor has never been actually isolated . . . , we have no means of telling whether the effect is not due to the combined action of a number of genes, any of which may in their

turn be responsible for other effects as well. Thus, if when using F a single gene is meant, the assumption is unwarranted; if a possible collection is meant, the notation is unwarranted. In case F or any other such ''character'' is sex-linked, however, (as F is in Morgan's formula), this representation is somewhat more defensible, for in this particular case the character always segregates completely as a unit in the heterozygous sex, and the integrity of the letter is destroyed only by mutation. It seems therefore permissible to use a single letter here, provided we call attention to the exceptional nature of the case. . . .

However, if the ''character'' may be made up of a collection of non-sex-linked genes, it should never be represented in this way, for then the various components need not all be coupled together as they are in sex-linked cases. The integrity of the letter is thus destroyable by their independent segregation, whereas the use of a single letter implies that in segregation its genic original acts as a unit. This objection applies to the M of the formula under discussion, even if we regard the formula as correct in intention.

To pursue the case to its conclusion, objection is to be raised to the insertion of MM into the formula also because it is provided in diploid quantity throughout ($\female$ = FFMM, $\male$ = FMM). The representation of any factor or factors which appear in like quantity throughout any complete series of cases, is superfluous. It is not legitimate to answer to this that the gene does not manifest itself in all these cases. Thus, in the case of the fly with the abnormal abdomen, let us assume, as is usual, that abnormality, the dominant, is also the presence. Then the abnormal fly is represented by AA or Aa, and the normal by aa. Normal is represented as the absence of abnormality, and yet for the production of the normal abdomen many factors, which together might be called NN, normality, are surely necessary. However, as these factors are present in all cases, they are not represented, even though they are in evidence as such only when A is absent and work differently or not at all in its presence (*i.e.*, do not manifest themselves), just as is the case with the hypothetical MM. If a certain somatic effect is produced, its representation in the germ cells is presupposed, and essential likeness of genic composition is assumed except where difference is indicated in the formula.

We have laid stress upon method of notation because a clear agreement as to method of symbolization is important for clarity of thought regarding the objects of the symbols. And as regards these objects, the genes, we hope we have indicated that, in regard to presence and absence and some other questions, it is necessary to conjecture freely and fully as to all the possibilities involved, if one wishes to avoid the paradoxical criticism—consequent upon the missteps which come inevitably to those who try to reach ''natural'' conclusions but avoid ''unbridled theorizing''—that one has actually indulged in wild speculations. . . .

THE AMERICAN SOCIETY OF ZOOLOGISTS
CHROMOSOME MOVEMENTS[1]

PROFESSOR C. E. McCLUNG

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THE substance of living things is unique, and marked by a peculiarity entirely lacking in the non-living—the power to reproduce itself in kind. In the operation of this process the unit of structure—the cell—is also the unit of function. The general details of cell reproduction are now well known and the importance of certain small elements, the chromosomes, fully recognized. These small bodies, approaching sometimes the limit of visibility, give every indication of being primarily responsible for the process of reproduction, because, before the cell can divide into two cells, each chromosome must accurately duplicate itself. This process is one of incalculable nicety and precision—the minute and definitely arranged subdivisions of almost molecular size each reproducing itself with greatest accuracy. The chromosomes and their parts have been found to correspond in their behavior most precisely with that of the characters represented in parents and offspring, so that biologists can many times predict the behavior of parental characters in their progeny. No element of structure or detail of movement is therefore without significance. A study of the chromosomes during their reproduction reveals many complicated movements, some of which suggest ready explanations, others being, as yet, without reasonable explanation. A plea is made that a more intensive study be undertaken of chromosome movements in the hope that a better understanding of them may lead to some explanation of the unknown processes of development and differentiation.

[1] Paper read by invitation before the New Orleans meeting of the American Society of Zoologists, December, 1931.

Why should time be given to the consideration of such a topic as this? Are not the chromosomes divided in the metaphase of mitosis and pulled to the poles of the spindle in the anaphase—and is not this the extent of their movements? A casual inspection of the literature might justify such questions, since rarely is there a full and critical discussion of the history of chromosome behavior. So long has attention been focused upon the striking appearance of the chromosomes in the mitotic figure that one almost inevitably visualizes them thus when they are mentioned. A tacit assumption that they are so limited appears in many discussions where the chromosomes are said to make their appearance by concentration out of a reticulum or even by some physical process of crystallization. There is occasion, it seems to me, to consider with care the manifold and exquisitely ordered transformations which manifest themselves with such bewildering detail in most cells.

There is something to excite wonder and admiration in the nicety and precision with which, on so small a stage, these little actors in life's drama play their so significant parts. Not only in their relatively larger manifestations are they interesting, but even in those details which approach the ultra-microscopic they intrigue us. The endless and innumerable repetitions of the same purposeful chromosomal program in organisms of high and low degree indicate most clearly the profound significance attaching to each detail and challenge our critical attention. Here, perhaps, lies concealed an explanation of those perennial biological problems of reproduction and differentiation. So much has been done to explain the relations of generations to each other by consulting the behavior of their germ cells that we are led to hope we may likewise decipher the cryptogram of their development. There must certainly be significance in each twist and turn of these fateful threads. They offer in their weavings in and out and in their comings and goings through the intricate dance of mitosis a basis

for attack upon that still unsolved mystery of differentiation in development. What is there, in a process obviously and perfectly designed to secure exact duplication of every minutest detail of the determining chromatin during mitosis, that will insure diversity in the resulting sister cells? This is indeed a paradox, but only so because we have failed to grasp the significance of some less obvious phenomena. If the chromatin is, as we have increasing reason to believe, the governing element in the cellular complex, then certainly a better understanding of all its changes must contribute to a solution of the problem of differentiation.

I regret that I do not bring you solutions, but only suggestions. It would be so satisfying if I could point out even some promising lines of approach to an understanding of the methods of differentiation in cells, but so far we wander in darkness. The most I can hope to do is to emphasize again the need for a reattack upon this basic problem. In its solution lies the hope of a much more complete understanding of all life phenomena, normal and pathological.

If we hope to get inspiration and help from what knowledge we have, the first step would seem to be the familiar one of analyzing and ordering the facts which we already possess. Accordingly, I will ask you to consider with me such information as we have regarding the form, extent and character of chromosome movements, because certainly within them will be found the key to our understanding of their significance.

It is so necessary, in the beginning, to conceive properly the physical characteristics of the chromosomes, the nature of their substance and the size of its aggregates, and the proportion of the parts, if we are to understand them. Until recent years brought a restudy of the living cell, general conceptions of the physical characteristics of the chromosomes were colored by the images seen in fixed material. It was difficult to imagine the solid threads of chromatin, thus revealed, as active, moving

bodies, although it was perfectly evident from a succession of stages that they must move. Actual observation of their activities and an experimental insight into their physical characteristics through microdissection, make appreciation of their real nature easier. There is now no difficulty in understanding the possibility of all the varied changes of which the highly labile chromatin gives ample evidence. But to explain why and how these manifestations occur is quite another matter. It can not be without significance that cells themselves are very small bodies and even in the largest of them the chromosomes are very minute objects.

Their subdivisions, or chromomeres, are near the limit of the visible, and must be approaching the molecular in size. These are the bodies for whose movements we seek explanation. They are so small that in a dead cell they would exhibit Brownian movement, but in the living condition, instead of this purposeless vibration, they show ordered and predictable changes. Because of the fact that our analysis of visible structures demonstrates that, even so far as we can see discrete elements, they are showing purposeful movements, we must reasonably conclude that they are continued to whatever final organized bodies there may be—let us say the molecules.

Ultimately, of course, whatever activities there are in the chromosomes, they must be molecular, but certainly these differ in some essential way from those of non-living matter—we say they differ essentially because the results are unique. If reproduction is the quality of living things which marks them most fundamentally in contrast to the non-living, and if chromatin is the substance which governs the activities of the vital material, then there is no escape from the conclusion that it must inaugurate the process. The conspicuous part it plays in the cells set apart for the preservation of the species strongly confirms this.

What the initial act may be in this apparently teleological behavior is beyond the reach of our direct obser-

vation, but an understanding of it is the goal of all biological investigation. In the absence of any positive information, we must make assumptions to serve as guides in the search for fuller knowledge. It is an exercise of the imagination which can do no harm and may lead to some comprehension of this profound mystery. The more we learn about the process of cell reproduction by mitosis the more apparent it seems that the chromatin can not long exist in a thread of a single series of chromomeres. Reports of splitting or duplication of the chromosomes in the telophase are frequent; of this act in the anaphase, occasional; and of its occurrence in the metaphase of the mitosis preceding separation in metakinesis rare, but apparently authentic. If such a condition were common, then it would seem that it is impossible for the chromatin units to exist in an unpaired state. Should this be the case it would indicate that the characteristic molecules must primarily be incapable of an unbalanced existence. This would do no violence to our knowledge of organic molecules in general, for valence must be satisfied. It would be necessary to assume only that the chromatin molecule had reached such a development that it must have, not only polarity, but also a bilateral symmetry.

There remains, of course, the crucial question of why, having attained such a balanced state, the chromatin molecule should destroy it through division. Various possibilities suggest themselves in answer. Thus, for instance, it might be that the number of atoms, electrons, negative charges, or whatever it is that constitutes molecules, can not exceed a certain number without breaking the bond with the proton, or other central element, of the system. A reversal of electrical polarity in certain elements might produce repulsion where attraction previously existed. Changes in other elements of the cell system might bring about division of the chromatin units, although in view of the apparently causal nature of the chromosomes in the cell, it is much more reason-

able to assume that reproductive activities originate within them.

Whatever the primary source of this reproductive activity may be, the visible effect is a movement of repulsion, separation or splitting of such a nature that the two resulting elements are practically identical. One is tempted to inquire whether the appearance of a mirror image relationship reflects an inner organization of this type. If so, it might be easier to understand the tendency or, indeed, the necessity, of the chromatin always to exist in a double thread state. We are so accustomed to a bilateral symmetry in animals that it would satisfy an intellectual conformism to find it existing in their ultimate control units. Polarity, so characteristic of organisms, certainly exists, and a bilateral arrangement is obvious, but whether this extends to the constituent molecules is, of course, beyond our observation.

An interpretation of the movements of chromosomes must be, as in all biological matters, in terms of cause and effect. Why are all these intricate and involved processes necessary? They seem to be principally concerned with only two of the general functions of protoplasm—metabolism and reproduction, and, in a final analysis, probably in the main with the latter. We must therefore seek for explanations of how chromosome movements facilitate reproduction. All our experimental observations seem to indicate that the substance of chromosomes is the governing agent in reproduction. We also note that, in any reproductive act, the chromatin is the first material involved. Before a cell can divide its chromosomes must be duplicated. As Roux long ago observed, the essential act here is the accurate duplication of the finest observable subdivision of the chromatin thread—the chromomere. Linear extension obviously provides the most favorable physical conditions for this almost molecular duplication of parts. The reason for such an act is now much more apparent in the concordance with genetical results than it was when Roux gave

his explanation for precision of mitotic division in respect to the chromatin. One form of the movement of chromosomes receives, therefore, a ready explanation in their reproductive act.

If the stretching out of the chromatin thread facilitates the subdivisions of its finer elements, so also does its contraction into the dense metaphase elements make easier the translocation of the chromatid to the poles of the spindle. The opposite acts of extension and contraction are of obvious mechanical advantage when their purposes are considered. Since they are of universal occurrence in plant and animal mitoses, they result from the common necessities of all cells.

Other types of chromosome behavior do not find so ready explanation. Why should it be necessary for the elongated chromosomes to "act like eels in a basket," as Strangeways describes them from the study of living cells.

In many spermatocytes there is a definite looping of the chromosomes in the peritene stage, but in those of other species this is lacking. Such behavior can not therefore be of the general significance which marks linear extension and contraction in all mitotic chromosomes. Even so it characterizes the spermatocytes of perhaps a majority of animal species and must represent some important function. Previous to this sweep about the nucleus in the peritene stage, the chromosomes have been arranged in the diatene with their ends attached at opposite poles of the nucleus, but in making the loop, one end detaches itself and comes to lie beside the opposite end—producing the so-called bouquet stage. A new form of polarity is thus set up, but the reason for it is not obvious.

From all the available facts it seems reasonable to conclude that the chromosomes are definite, discrete bodies, capable of automatic movements of a highly diversified character. These movements must be directly related, causally, to the activities of the cell, particularly to its

reproductive function. Pursued to a logical conclusion, this must mean that the ultimate units of structure, molecules if they be such, reproduce themselves as the primary step of a series of duplications which finally involve the cell as a whole. Many of the complicated changes of the chromosomes suggest at present no causal significance, but it is unreasonable to suppose that this is lacking. The difficulty is that we have as yet no clue to guide us in our search for the meaning.

Ultra-violet photographs of living cells resemble in almost every respect the pictures of fixed and stained preparations. The validity of the images we have so long studied is now beyond question. We may undertake the study of the intricate maneuvers of the chromosomes, secure in the belief that what we see in the succession of static pictures represents truly the continuous process of the living cell. Here is a problem of utmost theoretical significance, one to challenge the best of technical effort, accuracy of observation, and, above all, of constructive imagination. The deceptive ease of experimental method has attracted many to its use in the hope that thus might be reached a knowledge of the rôle of the chromosome in cell processes. Much as these new disciplines have added to our knowledge, they are no substitute for that painstaking, laborious, and cumulative method of observation and inference which has already given us most of what we know of the chromosomes. There is much need for more capable workers in the study of the eternal dance of the chromosomes in reproduction, and I hope that an appreciation of the possibilities here may lead to many accessions to the ranks of the observational cytologists.

ON THE TROPHIC IMPULSE SO-CALLED, ITS RATE AND NATURE[1]

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THE general subject of trophic impulses as well as of trophic nerves has long been one of uncertainty and obscurity. To the neurologist of a century ago nerves were either sensory or motor, but this simple classification was not to last long. In 1845 the Webers discovered, somewhat accidentally, that the stimulation of the vagus nerve did not induce the beat of the heart, but, on the contrary, checked the action of that organ. Thus ground was cleared for the idea of inhibitory nerves. Six years later, in 1851, Ludwig in his study of the salivary glands of the dog advanced strong evidence in favor of secretory nerves. In this way by gradual steps new categories of nerves were added to the original two.

As early as 1823 Mayo called attention to the fact that after cutting the trigeminal nerve the conjunctiva of the eye became inflamed, the cornea ulcerated and the face on the operated side edematous. These conditions were subsequently produced experimentally in lower animals by Toderà, Magendie and Longet. Although a number of workers openly disagreed with these results others confirmed them, and this confirmatory evidence together with much more of a generally similar character led several of these early investigators to conclude that beside the kinds of nerve-fibers already enumerated, trophic fibers must be added to the list. These fibers were believed to spread through the nerves of the body in association with sensory, motor and autonomic elements, and to be profoundly important in determining the metabolic activities of the tissues to which they were

[1] Paper read by invitation before the New Orleans meeting of the American Society of Zoologists, December, 1931.

distributed.   This idea was clearly and forcibly ex-
pressed by Samuel, who, in a volume entitled "Die
trophischen Nerven" published in 1860, declared that
"der Grund der Ernährung liegt in den Zellen, das Mass
der Ernährung in den trophischen Nerven."   Thus
Samuel may be regarded as the founder of the trophic-
nerve theory, a theory that met with a favorable recep-
tion from such distinguished investigators as Weir
Mitchell ('72) among the older workers and L. R. Müller
('24) among the moderns.

But it must not be thought that the theory of trophic
nerves was by any means generally accepted.   Virchow
('68), with his strong predilection for the independence
of the cell, opposed it vigorously, and in this he was fol-
fowed by Cohnheim ('82) among the earlier investigators
and more recently by Roux ('10), Mönckeberg ('15) and
Ricker ('24).   No one could deny after the strong con-
firmatory evidence brought forward by Claude Bernard
('68) that the cutting of the trigeminal nerve was ordi-
narily followed by serious consequence to the eye, but it
was believed by most students of the subject that these
consequences were due to other causes than the loss of
trophic fibers.   Both Snellen and Donders showed that
after severing the trigeminal the inflammation of the eye
could be postponed six to ten days if care was taken to
protect that organ against mechanical injury and the
irritation due to dust.   Thus it appeared that the re-
sultant panophthalmitis was not due to the loss of trophic
fibers so much as to the loss of sensibility, a loss which
prevented the ordinary protective and cleansing reflexes
of the eye.   Such an explanation, however, could not be
applied to muscles which, as is well known, atrophy after
the nerves that supply them have been cut.   This de-
generation, however, was commonly ascribed to loss of
normal function, in brief to inactivity, a state which is
usually associated with deficient circulation.   As a matter
of fact, a considerable number of investigators ascribed
all trophic disturbances to vasomotor maladjustments

resulting from nerve cutting. Such workers admitted the existence of trophic influences, but like many others saw no reason for assuming specific trophic fibers.

From the mass of conflicting evidence that this general problem has called forth and that has recently been very fully surveyed by Fleischhacker ('27), it is impossible to draw any safe conclusion. The most that can be done at the present juncture is to state in a rather categorical manner the various explanations that have been offered from time to time for those obvious conditions that have been grouped under the general head of trophic disturbances. These disturbances, according to the numerous investigators who have interested themselves in this field of research, have been assumed to be produced in one or more of the following ways: (1) By interference with the normal activity of true trophic nerve-fibers; (2) by interference with intercellular metabolic relations in which nerves are no more important than other types of tissue; (3) by interference with the local blood-supply usually through vasomotor irregularities; (4) by interference with peripheral sensibility whereby the normal protective reflexes are prevented; (5) by interference with normal functional activity resulting in inaction. The instance of trophic activity to be considered in this paper will be discussed from the standpoint of these five assumed explanations.

The lateral-line nerve of the common catfish, *Ameiurus nebulosus* (Les.), is a very convenient nerve for the study of trophic activities. It extends as a subcutaneous bundle of fibers from the rear of the head of the fish posteriorly over its flank nearly to its tail. In its passage from the head it diminishes in size by giving off small branches to two dozen or more lateral-line organs located in the lateral-line canal which extends, parallel with the nerve, along the side of the fish. This canal is immediately under the skin and opens freely to the exterior by pores which mark on the surface of the animal the so-called lateral line. The lateral-line organs are large

sense-buds located segmentally in the epithelial lining of the canal. As previous work has shown (Parker and Van Heusen, '17), they are receptors probably stimulated by movements of the surrounding water, including vibrations of low frequency.

For experimental purposes the lateral-line nerve is conveniently accessible immediately under the skin of the catfish in the region just below its dorsal fin. Here the nerve may be readily cut, after which the slight incision necessary for the operation quickly heals and the fish recovers. Brockelbank ('22, '25) has studied the effect on the lateral-line sense-buds of cutting this nerve. The buds anterior to the cut, that is on the proximal segment of the nerve, remain unaffected; those posterior to it gradually degenerate. On the fourth day after the operation, only slight traces of beginning disintegration can be seen in them. Their degeneration, however, may be complete in some instances in as early as seven days, in others not until thirty-five or more days. If the fish is allowed to live for some time after the operation, regeneration of the sense-buds invariably sets in. This may be well advanced, according to Brockelbank's observations, at fifty-four days after the cutting of the nerve and may be practically complete at a hundred and sixteen days. In these respects the lateral-line buds show a general agreement with taste-buds. Presumably as in the latter, the degeneration and regeneration of the sense-buds are dependent upon corresponding changes in the associated nerve; in other words, the nerve exhibits a profound trophic influence over the buds in that these receptors disappear with the degeneration of the nerve and reappear with its regeneration.

From the tables illustrating Brockelbank's paper, it is clear that the lateral-line buds do not degenerate and regenerate all at one time. There is some indication that in these operations a certain amount of sequence is followed. As shown in Table 1, the first bud posterior to the cut in different fishes was only partly degenerated at

4, 7 and 10 days, whereas, excepting at 21 days, it was completely degenerated at 13, 16 and 35 days. The second bud was, excepting at 7 days, only partly degenerated at 4 and 10 days, but completely so at 13, 16, 21 and 35 days. Thus degeneration must be assumed to be a relatively slow process in the catfish arriving at its completion only after some dozen days or so. If now the table is inspected for the sequence of degeneration, a certain amount of favorable evidence is to be seen. Thus at the four-day stage the ten buds which constitute the series that extended from anterior to posterior over the lateral line in a given fish were all in the condition of only partial degeneration. At the thirty-five-day stage the anterior part of the corresponding series is composed of nine completely degenerated buds followed by three partly degenerated buds and one fully degenerated. These conditions suggest that after the nerve is cut a wave of incomplete degeneration passes over the lateral-line organs marking the initial stages of this process and followed later by one of complete degeneration. It must be confessed that the rest of the table shows very little evidence either for or against this hypothesis, but the first and last of the records in this series are distinctly favorable to such a conclusion.

The degeneration of the lateral-line organs presumably follows the degeneration of the lateral-line nerve and if these organs degenerate in succession and not simultaneously, it is reasonable to expect that the nerve has done the same. The effect of cutting the lateral-line nerve on the severed part of the nerve itself has, therefore, been studied by Mrs. V. L. Paine and myself with the view of ascertaining the character of its degeneration. Does the lateral-line nerve after separation from its central connections degenerate progressively as the series of buds seems to indicate or does it disintegrate simultaneously throughout its length? The current opinion among the majority of students of nerve degeneration is to the effect that nerves, after severance from

their trophic centers, degenerate throughout their extent simultaneously. Of recent workers Bethe ('03) is the principal one who has opposed this opinion and who has advocated progressive degeneration in place of a simultaneous process.

To test this question on the catfish, we cut the lateral-line nerve on one side and studied its degeneration as indicated by the disintegration of its medullary sheath. When degeneration first set in it was impossible to distinguish any difference between the conditions of the nerve-fibers near the cut and at some five centimeters or so posterior to it. A comparison of these regions would have led to the conclusion that degeneration was simultaneous. When, however, two such regions were compared twelve to thirteen days after the operation, an unmistakable difference could be observed and it was clear that at that stage the region of the nerve near the cut was farther advanced in degeneration than that far from this point. This general condition was repeatedly observed in numerous preparations and was so manifestly present that we have no hesitation in concluding that the lateral-line nerve of this fish after some twelve days of separation from its trophic centers shows unmistakable evidence of progressive degeneration and that this degeneration spreads from the center toward the periphery. In this conclusion we agree completely with Bethe and are opposed to those workers on nerve degeneration, who claim that this process is strictly simultaneous.

The conclusion arrived at in the last paragraph is entirely consistent with what little is known of the degeneration of the lateral-line sense-buds. Both nerve and buds appear to degenerate progressively in that after cutting the nerve the anterior or proximal part of the system degenerates in advance of the posterior portion. It is interesting to note that the evidence for this state is drawn independently from the condition of the nerve and that of the buds. Furthermore, the evidence of its occurrence presents itself in the sense-buds and in the nerve

at almost precisely the same time, namely, at about twelve to thirteen days after the cutting of the nerve. At this stage the degeneration of the nerve and of the buds is in full progress.

The regeneration of the lateral-line sense-buds, as shown by Brockelbank's studies, is clearly a progressive one. This is well shown in Table 2 of her paper. But this process, which takes place between about the forty-fifth and sixty-fifth day after the operation, is not one which involves a process closely comparable with the degenerative one already discussed. It is the result of the outgrowth of new fibers from the central cut end of the nerve, an obviously progressive operation. This growth of new nerve substance is of course gradual and spreads peripherally (posteriorly). It is, therefore, not surprising that the regeneration of the sense-buds should take a corresponding course.

If the degeneration of the lateral-line nerve is a process that progresses over the nerve from the cut end peripherally instead of one that affects the whole length of the nerve simultaneously, it ought to be possible to obtain some measure of the rate of this progress. The idea of such a progressive change is doubtless what has been in the minds of those authors who in previous discussions of this subject have used the expression trophic impulse or metabolic impulse, both of which imply some relation to the nerve impulse or chain of events exhibited by a nerve in its normal activity of transmission. No one, so far as we are aware, has ever attempted to measure the rate of the nerve impulse on the lateral-line nerve of the catfish, but judging from measurements of such rates on the nerves of other cold-blooded vertebrates, it might be expected to be in the catfish not far from twenty-five meters per second. Is there any reason to suppose that rates of this order of magnitude occur in the passage of the so-called trophic impulses over such a nerve as that of the lateral line? For a test of this kind the lateral-line nerve is most satisfactory, for its func-

tional relations are such that the two kinds of influences that pass over it do so in opposite directions. Thus the normal sensory impulses transmitted by this nerve arise in the lateral-line sense-organs and travel thence anteriorly over the nerve to enter the brain in the region of the medulla oblongata. The trophic or metabolic influences on the other hand, as shown in this paper, progress from the central region of the nerve, presumably from its ganglion-cell-bodies, peripherally to the sense-buds. Thus the sensory impulses take a centripetal course and the trophic influences a centrifugal one. Hence the favorableness of this material for the measurement of the rate of the assumed trophic impulses.

To ascertain the rate at which the trophic disturbances pass over the lateral-line nerve of the catfish, we cut this nerve on one side of the fish and then at a later period on the other side. After some twelve days when extensive degeneration of the medullary sheaths had set in, preparations of both nerves were made at the same time and a degree of degeneration shown by the anterior or proximal region of the second nerve to be cut was sought for on the first nerve. These regions of agreement would be expected of course to occupy different positions on the two nerves, posterior on the nerve first cut as compared with the second. The antero-posterior distance between the two regions of agreement must represent the length of nerve traversed by the trophic disturbance, as represented by degeneration, in the interval between the time of the first and of the second cutting. Rather to our surprise this distance proved to be very short, and it was only when the time interval was three or four days that satisfactory comparisons could be made. On the basis of a number of such tests it was ascertained that the rate at which the trophic influence passed over the lateral-line nerve was an extremely low one, approximately two centimeters per day, a rate which at once puts this type of transmission entirely out of the category of true nerve impulses and into a class by itself. That this rate is

probably very much more rapid in warm-blooded vertebrates than in cold-blooded ones is seen from the fact that complete nerve degeneration occurs in mammals in three to four days whereas it requires five to six times that length of time in cold-blooded forms. Nevertheless, even in instances where this process appears to be most rapid it falls far behind the rate of ordinary nerve transmission. Thus the lowest rate for such transmission, and this is in the nerve-nets of animals like the sea-pansy, is only seventy to eighty millimeters per second. Such a rate, however, is approximately 320,000 times that of the trophic influence. Hence there is good reason to believe that nerve impulses and trophic transmissions are fundamentally distinct.

The nature of the trophic influence that passes thus slowly over the lateral-line nerve of the catfish can be stated only hypothetically. Its great slowness indicates that it is not to be compared with the nerve impulse proper. Interference with it, as already pointed out, may result in the degeneration on the one hand of the nerve itself and on the other of its appended sense-buds. Of the five explanatory hypotheses that have been offered at various times for such conditions in general that which seeks to explain them as due to the inactivity of the parts concerned can have no application to the present instance. For when the lateral-line nerve is cut, the sense-buds and the distal portion of the nerve itself are left undisturbed and nerve impulses must continue to originate in the buds and pass over the attached portion of the nerve as usual. Hence if normal activity were all that was necessary to keep the peripheral portion of this mechanism intact, it should, under such conditions, remain so. But as a matter of fact under these conditions, as has been clearly shown, it degenerates completely. Hence normal activity of itself is insufficient to maintain the integrity of the nerve fibers and appended sense-buds of the lateral-line system.

Nor is there any evidence that after cutting the lateral-line nerve disturbances in the reflex protective mechanism or in the vasomotor system ever set in, as is so abundantly seen when the trigeminal nerve to the vertebrate eye is severed. Such disturbances, moreover, would scarcely be expected to occur in the lateral-line system, for the ordinary sensory nerve fibers to this system and to the adjacent skin as well as its vasomotor fibers do not find their way hither over the lateral-line nerve but over the numerous spinal nerves on the flanks of the fish. Hence there is no reason to suppose from the anatomical disposition of the parts that cutting the lateral-line nerve would cause in any way disturbances in the protective or vasomotor mechanism, and in fact none is seen. We, therefore, conclude that the degeneration of the lateral-line sense-buds in consequence of cutting their nerve is in no way due to interference with protective or with vasomotor factors.

In all our work we have seen no reason to assume specific trophic fibers. The lateral-line nerve is composed of a remarkably homogeneous group of elements. They give no evidence whatever of falling into histological subgroups, and physiologically the nerve has never been shown to be anything other than a purely sensory system. It is our opinion that all its nerve fibers are strictly sensory. In respect to specific trophic fibers we agree with Verworn ('99) and with Bayliss ('24), both of whom after a general survey of the subject declared against them. All nerve-fibers are trophic and transmit trophic influences either in the same direction as they do nerve impulses or in the reverse. In this respect double conduction must be a usual and normal phenomenon. How this can be accomplished has already been suggested (Parker, '29a, '29b).

What the nature of the trophic influence sent over nerve-fibers is can not be stated definitely. That it is something that passes over the fibers with extreme slowness seems probable from the results of our measure-

ments. Such a rate suggests the percolation of material through nerve-fibers, small as their cross-sections may be, rather than the spread of ionic readjustments such as represent the true nerve impulse. The physical difficulties concerning such a percolation has already been pointed out by Hardy ('27), but as every histologist who has worked with fresh nerves and stains knows, solutions of such materials do make their way into the axis-cylinders of fibers at the nodes of Ranvier and percolate slowly along the core. We, therefore, are of opinion that the so-called trophic influence in nerves is due to a substance or substances that emanate probably from the nucleated portion of the neurone and make their way over the whole length of the nerve-fiber, at the slow rate which we have determined roughly for the lateral line of the catfish. These substances we believe to be essential to the normal metabolism of the nerve-fiber itself and directly or indirectly to that of such end-organs as the lateral-line buds. A similar opinion, at least so far as the last step of this process is concerned, has already been expressed in the case of the taste-buds and their nerves by Olmsted ('20) and by May ('25).

Our conclusion, based on what we have seen in the relation of the lateral-line nerve to its sense-buds, is that *all* nerves are *trophic nerves* and that their trophic influences are not limited to one neurone but are the means of binding nerve-elements together. In this way the receptor cells in sense-buds depend upon their associated nerve-fibers. The control emanates from the nucleated part of the neurone and spreads probably in the form of one or more hormone-like substances throughout the nervous unit in which it originated, binding the part of this unit together and bringing it into close organic relations with others. In this last respect we touch upon the humoral agencies in nervous action, nervous secretions and the like (Parker, '31), a general field long ago cultivated by Ramón-y-Cajal in his hypothesis of the chemical interaction of neurones.

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SPONGES AND BIOLOGY[1]

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IF the sponges could express themselves, as is the desire of so many of us to-day, I think a well-speaking sponge might address biologists somewhat in the following fashion: "I realize that we are not as widely known as some others and yet I feel that our family memoirs show we are not an uninteresting race. Dujardin, as early as 1838, when the cell generalization was in the making, wrote of our active amoeboid elements. Lieberkühn, in 1856, told the world that we had eggs, much like those of dog or man. At an appropriate time, when the evolution idea was acting like a fresh ferment, Haeckel, in 1872, described the beautiful series of forms grading up from simplicity to complexity, which the genius of the sponge race had realized in one of our tribes, the Calcarea. Oscar Schmidt had already begun ('62) his series of memoirs telling of our efforts, especially in the manufacturing of spicules, to present clearly this idea of evolution to the minds of men. These earlier recorders of our ways and our achievements have been followed by a line of modern biographers, led by F. E. Schulze and W. J. Sollas, all of whom testify to our interesting features. We are then not an unknown people.

"In writing of us, some biographers have been drawn by temperament or by their conceptions of what is most important to dwell especially on our cellular behavior. In this way they have made us admired, although perhaps at too great a distance, by the physiologists. Others, and the majority, like the great writers just mentioned, have been led to describe the *results* of our activities, the builded and finished structures, and in dealing

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[1] Paper read by invitation before the New Orleans meeting of the American Society of Zoologists, December, 1931.

with these wonders of morphology many have shown an elegant technique that is well worthy of us. It will be admitted that the human study of classification, in its search for evolutionary affinities, owes us much.''

Thus the sponges! Let us see what they really can do in the way of some cellular activities. And first, in the elaboration of reproductive bodies.

The observable facts lead us all to conclude that sponges may develop from eggs. For in the body of the parent we find a complete series of stages from egg to ciliated larva, and no indication that the larva is formed from any other body. Similarly, in certain monaxonid and hexactinellid sponges the observable facts, if any conclusion at all is to be drawn from them, lead with equal strength to the conclusion that a ciliated larva quite like that developed from an egg may sometimes be developed from a mass of undifferentiated mesenchyme cells, the so-called archaeocytes. This conclusion seems well established, since the facts are abundant, may easily be observed with precision and have been recorded in full by two observers for two groups (H. V. Wilson, '91, '94; I. Ijima, '01, '03), and in part by F. E. Schulze, unsurpassed in his knowledge of sponges ('04).

If we reason from these and other facts we must conclude that in the sponge cells, commonly now known as archaeocytes, we have elements in which there lie potentially all the characters of the species. If a cell of this kind is properly fed by follicular slave cells, it shows that it has the power to become an egg, which in due course becomes a larva and then a sponge. When, on the other hand, it combines, as a mere cell, with its fellows, the aggregation shows that it can do all, including the making of a larva, that the egg can. In some forms, the spongillids, the larva-forming power of the archaeocytes is exhibited only when they develop into eggs. For when they aggregate forming multicellular masses or gemmules, these bodies develop directly into sponges. The larva-forming power is here inhibited.

With increase of knowledge and improved technique we may hope some day to be able so to activate these cells as to turn them at will into eggs, sperm, larva-forming gemmules, gemmules with direct development or specialized tissue cells, into which, I may remark, they have also the power of transforming. No one as yet has been able to do this. Nevertheless, some progress, if not much, has, I think, been made toward this goal.

We have learned that if silicious sponges are kept in confinement they at once begin to adapt themselves to the unusual and unfavorable environment. The oscula and pores gradually close and the current of water passing through the body is reduced and finally stopped. All ordinary feeding and aeration of the interior is now at an end. In this state of danger what we would think of as a *"sauve qui peut"* policy is adopted. Organization is abandoned. Canals close and cells give up their specialized callings, as Metschnikoff first showed in 1879. A movement begins toward points that are presumably advantageous. Here cells accumulate and form solid masses of a relatively simple structure. Elsewhere death strikes the struggling mass of elements, the actions of which are physiologically reducible, it may be, to tropisms. We thus have left, in the best cases, a coherent body of skeleton and débris, lodged on and through which are many brightly colored compact little masses, often more or less spheroidal and in the neighborhood of a millimeter in diameter. They suggest at once the gemmules found in many localities at the end of summer scattered through the skeletal remnants of dead or dying spongillids. Like them, they are resistant, and when transferred to their natural environment they again, like gemmules, quickly transform into functional sponges.

What has been the actual cellular behavior that has led to the production of these regenerative masses? The process, in the gross one of simplification, has been studied by several (Maas, Müller, H. V. Wilson), but it still remains a promising field of research. The archaeo-

cytes are certainly leaders in what goes on. Since the process is linked in many ways to what happens when sponge cells are forcibly and suddenly separated and then allowed to reunite, I pass over it here. I would say, however, that what we already know shows that the simplification of the sponge may stop at more than one point before it has gone as far as it can in its downward course and be succeeded by an upward process of organization which leads again to the typical sponge structure (Wilson, '07a). It should be mentioned that the gross aspects of this process were described long ago in France by Laurent ('44), and in Germany by Lieberkühn ('57), both of whom observed that when sponges were kept in confinement they degenerated, leaving masses of tissue that had regenerative power. Their brief accounts (Müller, '11b), interesting as they were physiologically, had been lost sight of by 1907, when the phenomenon was redescribed as a new method by which sponges might be grown (H. V. Wilson, '07a). Otto Maas at about the same time ('06) had produced similar bodies in calcareous sponges and thought that they might be regenerative, as indeed he later found they were ('07).

The study of cell behavior in reducing sponges, leading to the formation of regenerative masses, very naturally led to more direct experimentation on the cells. And so I was led to find out in the summer of 1907, at the Beaufort Laboratory of the Bureau of Fisheries, that silicious sponges in the normal state can easily be broken up into their constituent cells which stream out into the water like clouds of echinoderm eggs, and, if mechanically aggregated and sown with a pipette on glass or a mollusk shell, will transform into functional sponges (Wilson, '07b, '11a). Some few years later I had the pleasure of learning that the same method is applicable to hydroids (Wilson, '11b). Many now in the past 20 years have used and amplified this method of growing sponges and hydroids. The experiment is easy and the result always rather pleasantly surprising.

But when the dissociated sponge cells assemble and form the regenerative mass, usually now designated as the reunition mass, what share, if any, does each kind of cell take in building the new sponge? This has proved to be a question not so easy to answer and the conclusions reached by the investigators have differed in some important details. There are indeed several conceivable answers to our question. Let us formulate these conceivable answers themselves as questions.

We may then ask: (1) Is there a permanent specification of cells in a sponge, possibly into epidermal cells, epithelial cells lining the canals, collar cells, cells that secrete spicules, cells that secrete the horny matter of the skeletal fibers, and into the other distinguishable kinds of cells in the sponge mesenchyme? And do these several kinds of cells, after dissociation, crawl back into their proper places in the reunition mass? Or (2) does a wide-spread, perhaps universal, process of dedifferentiation occur, the various kinds of cells resorbing their special differential cytoplasmic features and passing into a generalized embryonic state from which differentiation again starts out as from a group of blastomeres or a gemmule? Or (3) does rejuvenescence of another kind take place, the elementary, undifferentiated, proletarian cells, the so-called archaeocytes, engulfing and absorbing the more specialized elements, this process of phagocytosis gradually leading to the formation of a quasi-embryonic mass like a gemmule? Or (4) do the archaeocytes, without exercising the open brutality of phagocytosis, remain as the only persistent elements simply by inducing a condition, or perhaps merely assisting in the French sense at the oncome of a condition, in which other cells die and disappear quietly by cytolysis? Or (5) finally, is the whole phenomenon a composite of such processes?

I have recently ('30) gone over this matter with Dr. J. T. Penney. We find (1) that my original surmise, *viz.*, that a permanent and general cellular dedifferentiation

takes place, is not supported by the observable facts. Nor (2) is there any extensive phagocytosis. Nor (3) can it be said that the differentiated cells of the sponge return to their proper places resuming their former characters. It is true, we find, that the collar cells do this, a fact that has been affirmed (Huxley, '11) by some and denied by others (Müller, '11a; Galtsoff, '25), but they seem to be the only cells behaving in this way. The nucleolate mesenchyme cells, the so-called archaeocytes, evidently give rise anew, as in a gemmule, to many of the specialized cell groups. A hitherto overlooked, or misconceived, type of mesenchyme cell, one of much interest in considering regenerative phenomena, was discovered. Like the archaeocytes it is amoeboid but much more actively so. Unlike them it has, in the form (Microciona) studied, distinctive cytoplasmic granules and a characteristic, non-nucleolate nucleus. It can then be distinguished with certainty. These cells are abundant and very active. They quickly crowd to the surface of the reunition masses and there unite to form a protective covering, the permanent epidermis. The indications are that cells of the same kind form the lining epithelium of the canal system, a layer essentially like the epidermis. It seems very probable that such cells in the normal sponge body perform the function of quickly healing over a wound. Wounds are certainly very quickly covered over with new epidermis, and this we know is formed by amoeboid mesenchyme cells which come to the cut surface and there unite (H. V. Wilson, '10). These nonnucleolate mesenchyme cells are not totipotent like the archaeocytes. Nevertheless, they are regenerative but their regenerative power is limited. They can not do all, like the slower archaeocytes, but what they can do, they do very quickly. They suggest that there is more functional organization in the cell-community which we call a sponge than one would anticipate.

We may say, then, that in the formation of a new sponge from dissociated cells, unspecialized totipotent

elements, together with regenerative elements of specialized power, reestablish, as a new formation, the bulk of the sponge. Nevertheless, the collar cells, carried over from what we may call the parent, being on hand, are made use of as such. The method of restoration employed is a combination method, one well adapted to reestablish the sponge structure quickly.

The recorded data indicate that the same method is probably sometimes, *i.e.*, at a certain level, employed in the case of the reduction masses already mentioned, but that in these the process of reduction may go farther in the direction of establishing a homogeneous, "harmonious-equipotential" tissue. I thought at one time this was probably accomplished by fundamental cellular dedifferentiation, all the cells passing back into a simplified embryonic state and so surviving, and I still think this idea should be kept in mind in renewed research in this field. Others (Maas, '10; Müller, '11b) have, however, reached the conclusion that the homogeneous state is arrived at by phagocytosis, the persisting elements being certain amoeboid cells which devour the others. One would think that, if this is so, the eventually persisting cells would be the totipotent archaeocytes and Müller concluded that perhaps this was so in the case of Spongilla. If, however, Maas' tentative conclusion for the Calcarea is right, these cells are for the most part not archaeocytes but specialized tissue cells, *viz.*, pore cells, that have dedifferentiated into a morphological and physiological state like that of archaeocytes. This, in its emphasis on pore cells, which in Maas' forms, the Sycons, have not been found by every one, is of course surprising, and Maas admits the possibility that in the end the dedifferentiated pore cells may be devoured by the real primitives, the archaeocytes. The field, as can easily be imagined, bristles with technical difficulties as do related ones, where also the need is to follow the later history of particular kinds of cells which become dedifferenti-

ated, so far as to lose their distinctive morphological characters, and which unite syncytially.

In this sketch of methods of propagation in sponges we may note the following outstanding facts. (1) The cytoplasmic body of a sponge cell can not be looked on as a persistent unity, *i.e.*, a permanently individualized mass. Fusion with other cells is too frequent and too complete to allow of such a view. (2) Morphological dedifferentiation of cells occurs, but at any rate in some cases, that of the collar cells, for instance, it is not final but temporary, the distinctive features being regained. (3) It has yet to be proven that sponge cells ever dedifferentiate into a regenerative, embryonic condition, although the dissociation phenomena in hydroids lends some support to the view that this is possible. (4) Adaptive types of cellular behavior, which tend to insure survival, are called out under adverse conditions, as in the reduction and dissociation phenomena. (5) Certain cells are totipotent, egg-like as it were, and may hence develop in any of several directions, as in the case of archaeocytes, while other cells may have only a special though very quickly exercised regenerative power, as in the case of the non-nucleolate mesenchyme cells.

The retention by cells of their essential nature, even after complete morphological dedifferentiation, is illustrated, as said, by the dissociated collar cells. A parallel, not complete by any means, is found in the metamorphosis of the solid ciliated larva of our common silicious sponges. I have recently studied anew at the Naples Station this corner of comparative embryology, that has not been touched in a descriptive way for many years, and I find that the ciliated cells covering the surface of the larva are in some way drawn into the interior, losing their shape, distinctive granules and cilia and becoming syncytially interconnected with other cells. Out of this syncytium reemerge well-defined blocks of cytoplasm surrounding the original epithelial nuclei, and these become the collar cells. Yves Delage's discovery ('92) was,

then, in the main a real one, although there seems to be no reason for believing that the cytoplasm of the collar cell is the same mass that surrounded the nucleus of the epithelial cell. Delage, to be sure, described a process of temporary phagocytosis whereby this end was achieved. In this process larval epithelial cells were engulfed by amoeboids, in which they remained for a time in a symbiotic state but from which they were eventually freed to become the collar cells. I believe with Maas ('93) and Nöldeke ('94) that Delage was here in error and that what he saw was not symbiosis but serious internecine conflict, that is, the incorporation by amoebocytes of some of the epithelial cells, perhaps those that could not, for one reason or another, be used in morphogenesis, and the actual digestion of the nuclei, at any rate, of the ingested cells.

As to the main point, however, Delage was right and passing over for the present the question, how much of the cytoplasm of the epithelial cell goes into the collar cell, we may say that in a general way larval epithelium cells do become collar cells. A parallel is thus offered, although, as I have said, an incomplete one, to the behavior of the dissociated cells. For the larval cell after its period of submergence regains in some degree its original nature, coming out with an independent and somewhat elongated shape and a cilium. On the other hand, it gets what it did not have before, a collar, and there are in the cases I have studied (Mycale, syn. Esperella, Esperia) certain distinctive cytoplasmic characters which it does not recover. It reemerges then into active individual life like, but yet unlike, its old self.

I may incidentally add that I find that Goette was correct in concluding ('86) that the definitive epidermis of the sponge is formed from peripheral cells of the inner larval mass. Delage and Maas added to this knowledge, but in some way the original discovery by Goette has been nearly lost sight of (Nöldeke, '94).

I may close this sketch of events in the cellular life of sponges by considering for a moment the subject of embryogenic variation. It is now clear, and I seem to have been the first to point it out ('91, '94), that in the metamorphosis of the monaxonid sponge larva the flagellated chambers may be formed in two ways in the same species, indeed in the same local race, and perhaps even in the same individual. In the first place they are formed, as I have just said, from the larval epithelium cells that are drawn into the interior, strictly speaking, and not going beyond the known facts, from the nuclei of these cells and the cytoplasm which surrounds them after they have entered into the general syncytium. In my earlier work I observed this method but failed to discover the true origin of the little cells that group themselves around an intercellular space and transform into collar cells. Nevertheless, I recognized and recorded it as a method of chamber-formation distinct from the one I now turn to.

In this, the second, method the collar cells are formed from comparatively large mesenchyme elements, formative cells or archaeocytes, which divide. The two methods are sharply separated, far more so than I imagined when I first recorded them. For one is not derived from the other, as I had thought. Delage's discovery of the true nature of the first method makes this plain, and this method is clearly the basic ontogenetic one.

The two methods are practiced in the metamorphosis of other sponges than those for which I recorded them in 1891. Evans ('99), for instance, eight years later found both to occur in Spongilla. With this diversity of morphogenetic method established, a field perhaps is opened to experimental analysis.

The method of forming flagellated chambers from archaeocytes, while a secondary one in the metamorphosis, is used in a good many larvae. This is probably (Weltner, '07: 277) also the method of forming new chambers during ordinary post-larval growth, and is the method used in the development of a spongillid gemmule

and in some cases, at any rate, in regeneration from a reduction body (Maas, Müller). It is then the common method of later life, and its presence in the metamorphosing larva may be looked on as a precocious appearance. Why should it appear at this time? One can only speculate. It is clear that the larval epithelium cells have a hard time during metamorphosis. Some are lost at the surface, as I find occurs even in healthy larvae. Others are phagocytosed by amoeboids. The survivors form the chambers. Why such slaughter? Is it just that there are many more epithelial cells in the larva than are needed for the formation of chambers? However this may be, given the slaughter, it may well happen that sometimes it goes too far, and not enough epithelium cells (nuclei) are left for an adequate equipment of chambers. In such an emergency the archaeocytes are called on ahead of their time and they save the day. Their precocious coming into action may then provisionally be looked on as an adaptive measure carried out by the larva, an instance of that regulation of embryonic processes which insures that an attempt, at any rate, shall be made to form an embryo, if not in one way then in another. Cases of this regulative action more familiar to many are afforded by the spina bifida embryos of fish and amphibia.

This power of self-adaptation (self-regulation of W. Roux, '14) in an embryo is certainly one of the remarkable phenomena in nature. One can not but ask, although one can not answer the question, does it ever lead to hereditary change in embryogenic processes, to the development of a new hereditary way of modeling out an embryo from egg or mass of cells? To be sure, there are those who would say the power of self-adaptation is only an illusion, that in a case of this kind we have nothing more than a sequence of simple physico-chemical events, each mechanically necessitating that which follows, as when fallen leaves whirl about and collect in certain configurations. Which causes one to reflect that after all the

out-and-out mechanist is less realistic than he might be, for it really seems necessary at the present time to recognize self-adaptation as an actuality and advisable to learn both more cases and more about them.

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(Other references made in the body of the paper are included in the literature list given in Wilson and Penney, 1930).

GROWTH RATE AND HORMONE THRESHOLD WITH REFERENCE TO PHYSIOLOGY OF DEVELOPMENT[1]

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WILHELM ROUX ('95) defined the program of *Entwicklungsmechanik* ("physiology of development," as we should name it) as the resolution of developmental processes into simpler, but still complex, physiological components, and the analysis of the physiological components into really simple ones, "which may be identical with those which underlie inorganic or physico-chemical processes." This definition implies that nothing is left behind in the process of analysis, and that by summation or synthesis we could again reconstruct the whole from the parts. The implications of such a philosophy of biology are so profound that I have never felt satisfied to accept Roux's definition of the program; I prefer instead the definition that the aim of "physiology of development" is to discover the mechanisms of control of developmental processes. This sets us on the way to the discovery of mechanisms in development, but it does not imply a synthesis of development from the mechanisms; on the contrary, it definitely negatives such an attempt; and it reduces the status of physiology of development from "the most difficult problem which the human intellect has attempted to solve," as Roux characterized it, to a reasonable undertaking of a physiological order.

I shall present to you certain considerations involving hormone action in relation to growth rate as one of these mechanisms of control. Hormone action has been correctly conceived as organismal in the sense that all grow-

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ing parts of the organism must be equally exposed to its influence. It has, hence, been difficult to conceive how in development it could exert localized, mosaic-like effects; and yet this is a rather conspicuous feature—witness, for instance, the near-specific effect of the sex hormones! Although this had to be granted, yet when it came to the interpretation of the existence side by side of male and female characters in the same individual, as in gynandromorphs of birds, it was felt that an explanation on the basis of the action of sex hormones was almost necessarily excluded, and that some dislocation of genes in accordance with known mechanisms of gene action must be invoked.

It is readily shown that *intermittent* hormone action may produce very precisely localized mosaics of male and female patterns in growing feathers (Domm, '27; Juhn and Gustavson, '30). The purpose of the present paper is, however, to show that, in addition, even with constant action, a variety of similar mosaic effects can be produced, which are dependent on the principle of thresholds of reaction varying from part to part.

The French investigator Pézard ('22) was the first to lay emphasis on the principle of thresholds in hormone action, and he even used it to explain bilateral gynandromorphism in the plumage of birds, by the assumption that the right and left sides of the body in such cases possess different thresholds for the female hormone in the growing plumage. I regret that I overlooked this interesting theory of Pézard's in my recent paper on "Bilateral Gynandromorphism and Lateral Hemihypertrophy in Birds" ('31). Pézard, however, did not demonstrate that unequal thresholds existed in the plumage, nor did he attempt an analysis of the theory.

I. The first evidence that varying thresholds exist in the plumage of fowls and that the different thresholds possess a simple physiological basis came as a result of the work of Juhn and Gustavson in my laboratory

in 1930, and of their collaboration with Dr. Gwendolen Faulkner in 1931.

They showed that different feather tracts of the brown Leghorn fowl have inherently different growth rates, and exhibit correspondingly different thresholds of reaction to the female hormone. It was thus possible to produce birds with female feathers growing in the saddle region and male feathers in the breast region by keeping the amount of female hormone injected above the threshold of reaction of the saddle feathers, which have a lower rate of growth, and below the threshold of reaction of the breast feathers, which have a more rapid rate of growth. In the case of concentration above the thresholds of all growing feathers, an interruption of injections would record the reversion to the male type earlier in the breast than in the saddle, owing to reduction of concentration of the hormone in the blood first passing the threshold for the breast. If injections were renewed soon enough, the breast feathers became gynandromorph, while the saddle feathers remained pure female, owing to the fact that excretion had not reached the lower threshold level of the saddle before the new supply of hormone became effective. In all cases the threshold of reaction exhibited an increase in correspondence with increase of rates of growth.

II. In the individual feather the same principle holds (Lillie and Juhn, '32), and it can be applied to the explanation of some important principles of feather pattern. One of these principles comes out very prettily in the patterns that it is possible to produce in saddle and hackle feathers by injections of thyroxin. The saddle and neck hackle feathers of the brown Leghorn react to injection of c.p. thyroxin by deposition of black pigment, and by extension of barbule formation, which is normally confined to the bases of the barbs of these feathers, towards the apices of the barbs, and even to their extreme tips. In the case of the saddle feather, with a single injection of 0.5 mg of thyroxin into a capon weighing

1,800 grs, the pattern produced is a narrow black spindle centering on the rhachis of the feather. The spindle records at its apical end the first effective concentration of excess thyroxin in the blood; its widening records rise of concentration with absorption, and its taper to the basal end records excretion to normal. The threshold of reaction thus rises from the base of the barbs towards the apex. With a single injection of 1 mg the spindle is wider, inasmuch as successive apical thresholds are passed; the widest part of the spindle in this case is above its center, recording a more rapid process of absorption than of excretion. Single injections of 1.5, 5 and 10 mgs of thyroxin produce patterns that finally reach the margin of the feather, all threshold levels being exceeded in the last case. The pattern in the successive cases approaches and finally reaches the form of an inverted equilateral triangle, the process of absorption being recorded only by a slight anterior projection from the center of the base.

Repeated symmetrical patterns can be recorded on either saddle or neck hackle feather, by interrupted injections properly spaced. In the production of these patterns the invariable principle is that threshold of reaction rises from base to apex of the barbs.

Lillie and Juhn ('32) have shown in a very detailed way that this gradient of thresholds corresponds to a gradient of growth rates of the successive levels of the individual barbs. The apical portion of the barb, which is first formed in development, has the most rapid growth rate and this diminishes constantly towards the base.[2]

Patterns of similar form, though different in detail, may be produced by injections of female hormone. Thus two substances demonstrate equally well that the principle of a direct relation between rate of growth and

[2] This can not be adequately described without the figures which were used in the address. Those interested in this fundamental point in the physiology of development of the feather are referred to the paper by Lillie and Juhn ('32) published since this address.

threshold of reaction holds as well for parts of a single feather as it does for feathers of different growth rate.

We have spoken of feather patterns of axial origin dependent for their form on the principle of the relation between threshold of reaction and rate of growth. Another principle is, however, involved in the determination of the patterns, *viz.*, the principle of *rate of reaction*. The amount of effect produced in a given time is naturally greatest in regions of most rapid growth rate. Not only is this so, but the latent period before reaction begins, which is very considerable, ranging from 24 to 48 hours or more, is much shorter in regions of greatest growth rate. It may therefore happen in a case where the determining agent reaches a maximum concentration in a very brief space of time that its effect is confined to the margin of the feather. It is possible to produce such marginal marks experimentally. Indeed, any pattern produced by a determining agent operating at a concentration above all the thresholds must begin at the margin in point of time. The pattern produced in any case depends on the form of the curve of concentration with reference to the two principles of threshold and rate of action. Patterns may thus be of marginal as well as of axial origin.

III. Asymmetrical patterns also may be determined by differences in rates of growth on the two sides of the plane of symmetry. In the case of feathers of the wing-coverts we have been able to secure reaction to the female hormone confined to one side of the vane. It is interesting to note that on opposite sides of the body such asymmetries are mirror images of one another. The same principle appears to apply to bilateral gynandromorphism of the plumage of the entire bird; the writer (Lillie, '31) has shown that this condition is associated, frequently at least, with hemilateral hypertrophy, and that the male plumage is confined to the hypertrophied side. The assumption is that the hypertrophied side has a more rapid rate of growth with a correspondingly

higher threshold of reaction to the female hormone, so that, with the defective ovarian conditions obtaining in such cases, only the lower threshold of the more slowly growing side is attained by the hormone; the plumage of the other side would thus remain asexual, *i.e.*, male in type.

IV. Returning to our more general considerations, we can see how a simple physiological mechanism of control (such as a hormone circulating in the blood), operating on a developing system, the feather germ, which possesses gradients in growth rates, can produce astonishing variety of results in structure and pattern; and we can thus begin to understand one of the ways in which the almost infinite varieties of natural feather patterns may have been guided in their evolution. Other mechanisms of control of the feather type also exist in the genetic composition of feathers and in special innate properties of the different feather tracts, which constitute a mosaic of largely self-determined entities in other important respects. The physiological controls of which we have been speaking operate within limits set by these innate controls.

Two classes of phenomena are involved, *viz., threshold* and *reaction.* "Threshold" may be defined as quantity of condition necessary for occurrence of reaction. "Reaction" is the specific performance of a living system. These are related to rate of growth as follows:

(1) Increase of threshold is directly proportional to increase of rate.

(2) The latent period is inversely proportional, and the amount of reaction is directly proportional, to increase of rate; in other words, the time necessary for onset of reaction, *i.e.*, the latent period, is diminished, and the amount of reaction is increased with increase of rate.

Rate of growth is thus the determining factor (1) with reference to threshold, (2) with reference to duration of

the latent period, and (3) also with reference to the amount of reaction within a given period of time.

On the basis of ordinary physiological, or indeed chemical, principles it is easy to understand how the latent period should be cut down, and the amount of reaction within a given period of time be increased, with increase of growth rate. But it is not so easy to understand why a more slowly growing part of the system should react to a lower concentration of the hormone than more rapidly growing parts; in other words, have a lower threshold. This determination does not, however, stand alone.

In Child's experiments (*cf.* Child, '28) dealing with differential susceptibility along the axial gradient, disintegration in lethal solutions begins at the high point of the gradient and progresses towards the low point; in such an experiment the concentration is above the lethal point for all levels, and the result therefore measures merely the rate of reaction—in this case disintegration. This is in agreement with our results with supramaximal hormone concentrations. When, however, in Child's experiments lower concentrations of the lethal agent are employed, the phenomenon that he calls differential acclimation appears; disintegration proceeds in the opposite direction; and the region of highest metabolic rate survives and may subsequently regenerate the missing parts.

This phenomenon is primarily differential *survival* rather than acclimation, and the result is that the threshold of survival is least at lowest rates and greatest at highest rates, thus in the same order as our threshold of reaction.

The field of study of differential susceptibility to various agents—chemical, pharmacological, physical agents, radiation—is a very large one. These have been studied mostly from the point of view of injury rather than stimulation. It is accordingly difficult to find investigations comparable to our own. There is, however, rather general agreement that there is some connection between

differential susceptibilities and rates of metabolism. Packard ('31), for instance, says with reference to the biological effects of short radiations, after considering apparent exceptions to such a relationship: "These instances, however, are not sufficient to outweigh the large amount of evidence which indicates that there is some connection between susceptibility and the metabolic rate, or some physiological condition closely bound up with it." Wertheimer ('30) believes that there is a direct relation between rate of growth and susceptibility to thyroxin, young rapidly growing individuals being much less susceptible than mature slowly growing individuals, a relationship which is in the same order as our observations on the feather germ.

Rather general application of the principles of threshold and rate of reactions with reference to growth rates would appear to be possible in physiology of development. The distribution of growth rates within the organism varies enormously with age, with reference to organs and regions, and with reference to symmetry, whether axial or bilateral. These subjects have been much studied of late. If the principles of relation between growth rate and threshold apply to all vital reactions, they must be factors in effects produced by all organismal mechanisms of control, such as hormones and genes. In growth rate, therefore, we may have one of the most important mosaic principles of development.

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SHORTER ARTICLES AND DISCUSSION

AN INTERNAL BUT NON–GENETIC CHARACTER AFFECTING WING PRODUCTION IN RESPONSE TO LIGHT IN AN APHID[1]

A STRAIN of the rose and potato aphid *Macrosiphum solanifolii* (= *gei*) collected near Woods Hole, Massachusetts, in July, 1928, proved to differ from any other strain of this species which I have reared in exhibiting a considerable range of color in the females. Some females were bright green, some distinctly yellowish, with various gradations between. This was not an ordinary case of fluctuating variation, with bright green and yellow the extremes and the mode somewhere in the middle of the range. The intermediate colors were far too few to warrant such an assumption. There was a mode in the yellow part and another in the green part of the range, with a trough between.

This color difference was not usually apparent in the nymphal stages, and was never marked in them. Differences were distinctly observable in young adults, and often became greater with age. That is, a young adult yellower than the average would often, perhaps usually, become still yellower during its adult life. However, a female that was yellower than the average as a young adult was never found to turn distinctly green with age. More than fifty yellowish adults were individually observed during their lifetimes with respect to this point, and most of them turned somewhat yellower, some of them became much yellower, but none became more green. The comparisons were made with Ridgway's "Color Standards and Nomenclature," as the objective standard, and all the changes observed in these fifty or more aphids were toward the greater wave-lengths.

Similar tests were made with young adults that were bright green. In them, the changes occurring with advancing age were much less marked than among the yellow females, and few of them showed any very appreciable change at all. What modifications did take place were, however, toward the green. Now and then a very old female, at a time when the body for some reason becomes somewhat shriveled, was distinctly blue-green. I

am not, however, inclined to regard this change toward the shorter wave-lengths as the same phenomenon as the small change occurring in a few of the plump females toward greener color, since such old blue-green individuals have been repeatedly observed in other strains.

The comparison just made could lead to the conclusion that bright green was the standard condition, from which a change toward yellow was rather readily made. A diminution of the yellow was, however, difficult and never very extensive.

The possibility that the color difference had a genetic foundation was tested by rearing more than twenty bright green females and an equal number of distinctly yellow ones, and recording the colors of their progenies. These were recorded by their Ridgway names at first, then converted into their numerical or other symbolic designations according to the Ridgway scheme. Only their numerical values need be used here, since these values (from 1 to 72) are the ones that relate to wave-length. Only healthy adult offspring were studied, the few that were rejected being rejected wholly on other grounds than color. All were among the first four days' output of their respective parents. All were taken at approximately the same age, which was about four days after becoming adult. Experience has taught that at that age any color differences that would arise were already marked, and using adults no older than about four days avoided confusion of their color peculiarities with color changes (like the acquisition of blue-green by old females mentioned above) due solely to old age.

The progeny of the twenty yellow females had a mean numerical color rating (relating to wave-length) of 26.93 ± .065, while the progeny of the twenty bright green parents had a mean color rating of 27.01 ± .059. The latter group was a little greener, but the difference was not quite equal to its probable error. There is probably no genetic difference, therefore, between the yellow and bright green aphids.

The response of these two types of aphids to light and darkness, with reference to wing production, was tested by rearing a group of each kind in continuous light, and an equal number in alternating light and darkness, after the manner of my earlier experiments (Shull, '28, '29). The length of the light period was 8 hours, the dark period 16 hours. Their progeny are represented in Table I.

TABLE I

Bright green parents				Yellowish parents			
Continuous light		Alternating light and darkness		Continuous light		Alternating light and darkness	
Wingless	Winged	Wingless	Winged	Wingless	Winged	Wingless	Winged
35	100	158	120	69	80	107	70
Per cent. winged	74.1		43.2		53.7		39.5

Owing to the very erratic occurrence of wings in aphids, it is not safe to draw conclusions from such small numbers as Table I includes. In obtaining larger numbers it was not possible, along with other experiments in progress that time, to continue all four of the lines represented in that table, so only those two which showed the least contrast in the first experiment were repeated. These were the groups reared in alternating light and darkness, and for them the experiment was repeated four times. The light and dark periods were this time necessarily slightly irregular, but were the same for both green and yellow females. The light period averaged about 7 hours, the dark period 17 hours. Table II shows the results of these four experiments.

TABLE II

Bright green parents			Yellowish parents		
Wingless	Winged	Per cent. winged	Wingless	Winged	Per cent. winged
184	66	26.4	283	89	23.9
72	111	60.7	88	112	56.0
163	129	44.2	115	69	37.5
141	139	49.6	163	80	32.9
560	445	44.3	649	350	35.0

While only one of these experiments shows a markedly different response in the offspring of green and yellow parents, the difference is in every case in the same direction. It appears safe to conclude that the green parents produced more winged offspring than did the yellow parents. Since the color was not of genetic origin, there must have been some physiological modification of a more or less permanent nature so far as the

individual was concerned. As pointed out above, it is somewhat more in keeping with the evidence to suppose that the yellow individuals were modified greens than that the greens were modified yellows. Yellowing, according to this supposition, was a condition which could easily increase in degree, but was not likely to disappear in a given individual once it got a start.

The nature of this modification is wholly unknown. It could be an infection, but there is no evidence of the presence of any other organism. The work of Ackerman (1926) on the physiology of wing development opens up many possibilities for speculation, but the present experiments offer no support for any proposed view. The fact that there was a modification seems, however, to be established.

<div align="right">A. Franklin Shull</div>

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AUTOPLASTIC TRANSPLANTATION OF GUINEA–PIG SKIN BETWEEN REGIONS WITH DIFFERENT CHARACTERS

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At the suggestion of Professor S. Wright, experiments involving autoplastic transplantation of guinea-pig skin were undertaken to determine whether pigmentation, length and direction of growth of guinea-pig hair persist in a new environment or whether there is modification of these factors to conform to the surrounding pattern. Most of the animals used in the experiments showed spotting effects, and the technique consisted of an interchange of areas of skin of different color.

Although there are many records of skin transplantation, there are few cases in which it has been employed as a genetic method. Carnot and Deflandre ('96) and Leo Loeb ('97) found that black skin, transplanted in place of white on the ear, per-

sists, but that white, transplanted to a black area, became pigmented after a short period. This was due, according to Loeb, to migration of pigment granules from the surrounding black area into the graft skin. This was indicated by the fact that black skin transplanted to a white ear became paler for a time, while the surrounding area became pigmented. Their observations were restricted to changes in pigmentation of transplanted skin and were not concerned with the color of the hair.

Danforth and Foster ('29) used the method of skin transplantation in the fowl to determine the mode of action of genetic factors on the activity of the feather follicle. They found that color pattern of the feathers in the transplanted skin continues to be like that of the donor if the host is of the same sex. If of the opposite sex, the feathers became like those of individuals of the donor breed of the sex of the host. The hen feathering of male Sebrights, however, turned out to depend on the reaction of a local factor to the male hormone.

Their evidence also indicates that the difference in the rate of feathering in young Leghorns (rapid) and Rhode Island Reds (slow) is determined almost entirely by factors residing in the skin. Reciprocal grafts of the two breeds show that the time of the first production of contour feathers is constant for each breed and is not changed by transplantation. In the case of Plymouth Rocks, both local and correlative factors were indicated.

There are seven known series of allelomorphic factors acting on coat color in the guinea-pig (Wright '27). There was variation in five of the seven series of factors among the animals used in these experiments. Three of the series $(S, s; A, a; E, e^p, e)$ effect color pattern, while the other series $(C, c^k, c^d, c^r,$

TABLE I

Pattern			Intensity	
S	s	•	Black series (E)	Red series (e)
EA Agouti	Agouti-white	CP	Intense black	Intense red
e^pA Agouti-red	Agouti-red-white			
eA Red	Red-white	Cp	Pale sepia	Intense red
		c^kP	Sepia	Yellow
Ea Black	Black-white	c^kp	Very pale sepia	Yellow
e^pa Black-red	Black-red-white			
ea Red	Red-white			

c^a; P, p) modify the intensity of the color in the pattern. Table I shows the factors concerned with the regulation of the color pattern and intensity of the color of the hair of engrafted areas.

The spotting factor s, which acts to limit the colored areas to spots on a white background, was present in most of the animals used. A spotting pattern of black and yellow (within the colored areas) was often present, the intermediate allelomorph e^p of the series E, e^p, e being present in such cases. This series differentiates between black and yellow colored portions of the coat, some grade of yellow resulting if the animal carries e instead of E. The factor A is responsible for the agouti pattern in the dark areas of the coat, while if a is present instead, hairs in these regions do not show the agouti band.

The other two series of factors are concerned with modification of the intensity of the colors. The albino series (C, c^k, c^d, c^r, c^a) acts on both black and yellow, the allelomorphs of C reducing black to various grades of sepia and reducing red to yellow, cream, or white. The factor c^k was present in the sepia and pale sepia animals used, as well as in the yellow animals. The factor p also reduces black to sepia but has no effect on red.

Operations were carried out on young guinea-pigs, forty to fifty days old. The transplantations were all autoplastic and consisted of a transfer of rectangular areas of skin of approximately two square centimeters. The operation site was clipped, shaved and sterilized with 70 per cent. alcohol. The procedure in each case was that of an interchange of areas of skin of different color patterns. The grafts were held in place with narrow strips of adhesive tape, which was found to be as satisfactory as sewing the grafts in place. It was essential to hold the graft tightly in position, and this was accomplished by taping a small rubber sponge wrapped with sterile gauze in place over the graft. The graft was usually sufficiently incorporated within ten to fifteen days that the bandage could be removed.

It is seen from Table II that pigmentation of the hair was not modified in fifty-two successful grafts. The grafts were in every case observed for a long enough period as to leave little doubt that the color pattern is persistent. The animals were kept under observation for periods of between six and nine months, and there is very little possibility that observation for a longer period would have yielded different results.

TABLE II

		Type of transplant	Successful grafts	Color pattern unmodified
Color on white	Black series	Agouti on white	1	
		Black on white	4	
		Pale sepia on white	3	8
	Red series	Red on white	7	
		Yellow on white	1	8
White on color	Black series	White on agouti	2	
		White on black	4	
		White on pale sepia	2	
		White on sepia	2	10
	Red series	White on red	5	5
Dark color on red or yellow		Agouti on red	1	
		Sepia on red	1	
		Black on red	1	
		Sepia on yellow	1	4
Red on dark color		Red on black	1	1
Agouti		Back to belly	10	
		Belly to back	6	16

Four general types of transplants were made to test the persistence of color pattern. Transplantation of skin from a colored area (whether of the black or yellow series) to a white area showed that the hair follicles do not lose their pigment producing potentialities in an unpigmented area. Sixteen grafts of different color pattern, involving both red and black series, to a white area were all in agreement. L. Loeb reported that in cases in which black skin was transplanted to a white area there was a considerable increase in size of the black area due to migration of pigment into the surrounding white area. We did not observe any change in the area surrounding the grafts in any case.

The second type of transplant was that of white skin to a colored area. Even this type of graft, which was thought most likely to be subject to change, did not acquire a pigmentation after transplantation. Fifteen white grafts in a colored area indicated that white hair does not become pigmented. Accord-

ing to L. Loeb and earlier workers, white skin rapidly acquired a pigmentation when transplanted to a dark region, in some cases becoming indistinguishable from the surroundings. While some of our grafts of this type did become slightly darker along the border after four or six months, there were many cases in which such grafts remained unpigmented throughout the period of observation. In no case did the hair become pigmented.

Reciprocal transplants of areas of the red and black series of colors indicated that the original color is maintained in an area of different color.

The agouti guinea-pig shows a marked regional difference in color pattern parallel to a pattern in length of the hair found in guinea-pigs of all colors. Each hair of the back is black with an intense yellow, narrow, sub-terminal band, while each belly hair has a wide, terminal yellow band. There is a very distinct difference in length of the hair of the two regions, the hair of the back measuring 27–35 mm in length in comparison to that of the belly, which is 10–15 mm long. We interchanged pieces of skin of these two regions in order to determine whether these differences are persistent. The new hair from all grafts from back to belly (10 cases) grew conspicuously more rapidly than those from the surrounding shaven region and reached the characteristic length and color of back hair. All grafts from belly to back (6 cases) produced short, light-colored belly hair, markedly in contrast with the surroundings. The differences remained unchanged as long as observed (at least six months). The differences of the hair of the two regions are so striking as to leave no doubt of the persistence of the hair characters.

After having examined a number of grafts, we noted the constancy with which the direction of the graft hair agreed with the direction of the host hair. Since hair direction in the guinea-pig is affected by well-known gene differences, the question of maintenance of direction in a new environment is of interest.

Unfortunately, we had failed to record the orientation of the graft in the operations already performed, but thought it improbable that the graft should, in every case, have been oriented so that the direction of the hair coincided with that of the host. In the experiments that followed, we reversed the grafts 180° so that the hair of the graft would be growing in opposite direction to that of the surroundings. In these cases, we kept the surrounding area clipped so that there would not be the factor of pressure to influence the growth of the graft hair.

The results obtained are not strongly in favor of either conformity to the original direction, or regulation to the new environment. The tendency seemed to be for the hair to stand straight up. There were two cases out of the eighteen that showed definite regulation. One was a transplant of red skin to a black area in which the transplant was reversed 180°. The other case was a transplant of smooth, belly hair of a rough agouti animal to the region of a rosette on the back, the transplant being reversed 180°. There was a marked tendency for the hair of the graft to be arranged in a whorl to conform to the direction of the surrounding hair. In the sixteen other cases in which the graft was reversed, the hair either stood almost erect or exhibited a slight tendency toward its original direction.

The results of these experiments definitely indicate the persistence of color pattern and length of guinea-pig hair following autoplastic transplantation. The question of the maintenance of direction of hair growth is not definitely settled, two cases out of eighteen indicate regulation, the others exhibiting a tendency for the hair to stand erect rather than to show either persistence or regulation.[1]

The tendency toward persistence of the characters of the graft seems to indicate that the differences in these characters are determined entirely by permanent cell differentiation, chromosomal or otherwise. It is to be noted that these grafts involve transplantation of two different types of pattern, one type being highly irregular and the other regular. The irregular patterns are those produced by the spotting factors s and e^p, the spots of the different colors being very irregular in size and position on the body. The pattern of the agouti animals, on the other hand, is quite regular, the markedly different patterns of back and belly being bounded by a sharply defined, symmetrically placed line.

It is tempting to suppose that the persistent differentiation produced by the spotting factors is due to somatic mutation. If spotting effects are due to controlled somatic mutation, these

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[1] Since this paper was written, Trotter and Dawson have published an account of experiments in which they reversed areas of guinea-pig skin, *in situ*, and found that in every case the graft hair maintained its original direction, even though the surrounding hair was allowed to grow. We have no explanation to offer for the partial disagreement of our results.

results suggest that such mutations are irreversible changes not affected by later correlative influences.

The regular pattern of the agouti animals has also been shown to be persistent. The manner in which the genetic differentiation of the two regions is brought about is unknown, the data indicating merely that the regional differences, both in color and length, are permanent within the skin itself. If somatic mutation is the correct interpretation of the differentiation in this case, it must be much more completely under control of the developmental pattern than in the cases of the irregular piebald and tortoise shell patterns.

<div align="right">

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AUSTRALIAN FRESH-WATER FISHES

The few distinctive fresh-water fishes of Australia are sufficiently unlike them to have, at first thought, little place in consideration of those of the other major modern land masses. The dominant Ostariophycine group (characins, catfish, carps) is represented in Australian fresh waters by a very few species only, belonging to two estuarine groups of catfishes. We have in Australian fresh waters the relic primitive *Galaxias* and *Ceratodus* (lung-fish), with relatives in each of the southern continents, from which one may argue southern land-connection and distribution for such forms at some point in pretertiary time. We have a genus of the typically marine Apogonidae, *Glossamia,* adapted to fresh water, and in Tasmania the peculiar cod-like blackfish, *Gadopsis,* without known relationships.

Of particular interest are small fishes allied to the marine silversides (Atherinidae), which, lacking normal competitors, have

seemingly entered fresh water and there developed several pecu-
liar divergent forms.   One of these, *Melanotaenia,* suggests laby-
rinth fishes of southern Asia in body and fin form, and has pun-
gent perch-like spines in dorsal and anal fin.   Another, the
blue-eye, *Pseudomugil,* is suggestive of certain carps and chara-
cins in appearance.

If one concede that Australia was separated from Asia
throughout the Tertiary, absence of continental fresh-water
groups there is evidence that these are of Tertiary origin and
distribution, a conclusion already indicated on other grounds.
That estuarine catfishes, established in Australian fresh waters
with a comparatively open field, show no marked divergent
specialization would indicate them to be a recent, not an early
trend of catfishes, and to have played little part in the general
world distribution of the catfish group by bridging areas of sea.

This discussion is incident to the examination of a small Aus-
tralian collection, which Mr. Raven obtained for the museum
several years ago, but which has only recently been studied in
detail.   We have found no previously published illustration of
the handsome characteristically marked *Craterocephalus stercus-
muscarum.*   Incidentally, McCulloch's checklist of New South
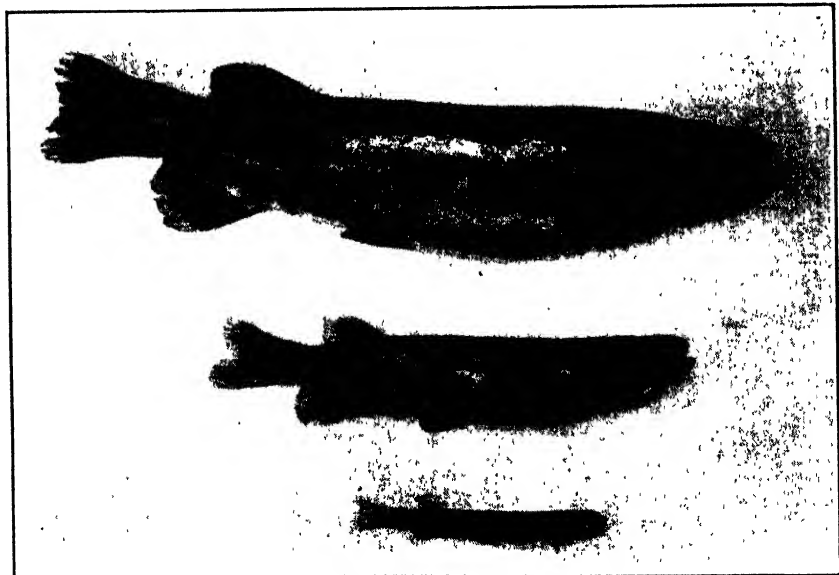
FIG. 1.   Galaxias.   Three stages of growth of specimens from the Arthur
River, Tasmania.

FIG. 2.   Just beyond the tent is the little stream, a branch of Babinda Creek, from which the specimens were taken.   In flood it covers the foreground to the center of the picture.

Wales fishes in the ''Australian Zoologist'' (1920–1922) is very fully illustrated, and a serviceable key to Australian fishes, both fresh-water and marine.

Most of the fishes comprising the above-mentioned collection were obtained in North Queensland, from a little stream arising on the slopes of Mount Bartle Frere, just south of the Bellenden Kerr Range, at a point where the stream emerges from densely forested foothills.   About half a mile farther north this stream is confluent with Babinda Creek, which in turn is a tributary of the Russell River.   All the fishes were secured by means of a minnow seine during the latter half of October, 1921.

The environment of this part of Australia has more in common with New Guinea than any other part of the continent. The Russell River system drains much of the coastal slope of the Cape York Peninsula between Cairns and Cardwell on the Pacific side.   This region, eighty-five miles long by twenty miles wide, is the only part of Australia with a rainfall of more than 100 inches per annum.[1]   Here, too, it is not very uncommon to record a rainfall of more than twenty inches in twenty-four hours.

[1] Taylor, Griffith, ''The Australian Environment,'' p. 115.   1918.

This region of heavy rainfall is for the most part covered with tropical rainforest, the vegetation having much more in common with that of New Guinea than with immediately adjacent parts of Queensland. These conditions of rainfall and vegetation have apparently restricted many of the higher vertebrates to this limited area and kept still other widely distributed Australian forms out of this specialized zone. This region of heavy rain-
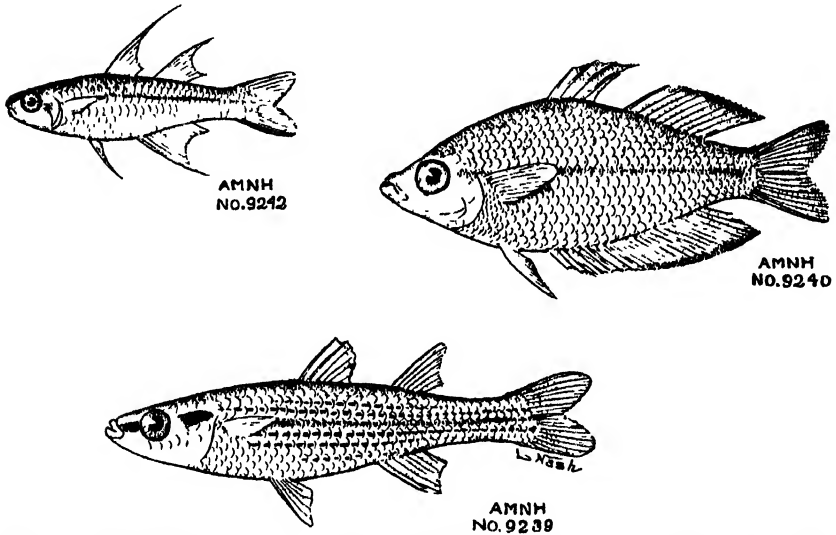
Fig. 3. *Pseudomugil* (upper left), *Melanotaenia* (upper right) and *Craterocephalus stercusmuscarum* (lower).

fall doubtlessly furnishes an abundance of food to the fishes of the streams, and it would be interesting to compare the forms inhabiting these swift streams of the well-watered area with those of streams of the drier parts of eastern Australia.

The atherinids in the collection were by far the most numerous fishes near Babinda and were commonly seen in small shoals, whereas the catfish were apparently not gregarious. The galaxias, which were obtained from the Arve and the Arthur Rivers of Tasmania, have somewhat the habits of trout but are much less active.

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LETHAL MUTATIONS AND DEFICIENCIES PRODUCED IN THE X-CHROMOSOME OF *DROSOPHILA MELANOGASTER* BY X-RADIATION

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INTRODUCTION

IN making a study of the effects of breakage in the X-chromosome of *Drosophila* for the purpose of detecting a possible sex factor, the writer has found, incidentally, many cases of so-called deficiencies of small magnitude that are lethal in the male. It is the object of this paper to present the main facts observed, pending a more intensive study of the subject by other workers in the laboratory.

In order to obtain such cases the writer has developed the following method. Either the wild-type or the Theta fly (usually the male) was irradiated and then crossed to an individual having an X-chromosome marked with several recessive mutant genes. Any mutation, whether lethal or not, that may have been induced at one (or more) of the loci of the normal allelomorphs of the mutant markers can be detected in the F_1 fly that has inherited the affected chromosome and its marked partner. Breeding tests will show whether or not the variation is associated with a lethal effect, or is simply a point mutation. If the variant female gives the two expected classes of non-crossover sons in about equal numbers, the case represents a point mutation, but if she gives only the class of sons that received the marked or untreated X-chromo-

some, the case then represents some type of "lethal mutation." In this paper we shall use the general term mutation for both kinds of cases.

By crossing treated flies to various stocks with different combinations of recessive sex-linked mutant genes, one can obtain both kinds of mutations at practically all the known loci. In the case of lethal mutations, it was found that the deficiencies varied in extent from cases involving a single locus to cases in which several adjacent loci were included. The more extensive deletions will not be considered in this paper, nor will the mosaics and gynandromorphs be discussed.

Number of Mutations Found

In Table 1 are listed 230 cases of mutations obtained by this method. These occurred at 15 of the principal loci that had been marked by recessive genes in the

TABLE 1

Loci	y	sc	w	w-N	N	ec	cv	ct	sn	lz	v	m	wy	g	pl	f	car	Totals	Mottleds involving in loci of white
Lethals	16	1	20	21	19	0	3	2	.		2	15	0	4	1	5	3	112	
Mutations	2	0	4	0	0	1	0	1	.		0	2	0	3	0	3	0	16	
Sterile or no test	12	1	10	10	23	0	0	0	3	1	3	36	1	1	0	1	0	102	
Totals	30	2	34	31	42	1	3	3	3	1	5	53	1	8	1	9	3	230	32

untreated X-chromosome. The 15 mutant genes were those for yellow, scute, white, echinus, crossveinless, cut, singed, lozenge, vermilion, miniature, wavy, garnet, pleated, forked and carnation. In addition to these, the table includes all cases found at the unmarked locus of facet, for a deficiency at this point is revealed in the F_1 female in the form of notched wings. Since notch acts as a dominant in a fly heterozygous for the deficiency, such cases are easily detected. There are also listed, in the last column of the table, 32 cases of mottled-eyed flies. We shall not consider the mottleds, except to say that in each case the locus of white was involved and in several

of them neighboring loci were also included, especially that of facet (notch).

The 230 cases are listed under three headings: (1) Lethals, or cases in which the male zygote receiving the treated X-chromosome does not survive; (2) point mutations that bred true; (3) sterile or untested flies. There are included in the last group a few variant females that were lost or died before a test could be made. As may be seen from the table, 128 females were fertile, and of this number 112 showed from the breeding tests that they were lethal in the male and only 16 bred true for the observed mutation. This gives a ratio of lethal to non-lethal mutations of approximately seven to one (87.5 per cent. to 12.5 per cent.). It is probably true that most of the females listed in the sterile group also carried lethals.

NUMBER OF MUTATIONS AT DIFFERENT LOCI

The number of mutations occurring at the different loci varies greatly, from single cases at echinus, lozenge, wavy and pleated to 53 at the locus of miniature. However, if we count both the white-notched and notched flies, this class leads with a total of 73. This raises the question as to whether the rate of mutation at the several marked loci actually varies as much as is indicated by the number of cases shown in the last horizontal line in the table. As previous work has shown, the rate of mutation is directly proportional to the dosage employed in giving the treatments. While the rate could be calculated, so far as the variation in dosage is concerned, yet in order to do this, it would be necessary to know exactly how many flies had been examined. In such a large scale experiment, conducted for another purpose, it was found to be impracticable to count and record all non-variant flies that were passed in review, although the approximate number of such flies can be determined from the number of culture used, a record of which was made.

There are several other factors that make it difficult to compare from the data the rates of mutation at the sev-

eral loci. The number of flies developed from some of the combinations was too small to give adequate data as to this rate. This is true for such loci as echinus, cut and carnation. Furthermore, a mutation at certain loci, especially if it is lethal, may affect adversely the viability of a fly heterozygous for a deficiency, and consequently, fewer variant flies would appear in the F_1 generation than would be the case where no such effect occurs. One such case that illustrates this kind of effect has already been found and described (Patterson, '31). The case in question involves a "gene for viability" near the left end of the X-chromosome, situated at some point lying between the loci of scute and prune. It was found that if a piece of the left end containing this postulated gene is missing, a zygote heterozygous for the deficiency is not viable. If the break producing a deficiency occurs between the locus of scute and that of the viability gene and the region of yellow and scute are eliminated, the zygote will survive; or if a deletion occurs to the right of the locus of the viability gene and eliminates such loci as white facet, echinus, etc., the zygote is also viable. Again, in case the treated individual was a Theta fly, mutations at the loci yellow and scute can not be detected in the F_1 female, because the X-chromosome of this stock has a duplicated fragment attached to its extreme right end, and this fragment has the normal allelomorphs for yellow, scute and broad. In view of these facts we shall not be able to determine whether the rate of mutation varies appreciably at the different marked loci.

We may now consider briefly the mutations that were found at the different loci. There were 30 females that showed a mutation at yellow. Eight of these were derived from cultures in which the wild-type fly had been x-rayed, and twenty-two from cultures in which the treated X contained an inversion. The latter stock, which is known as scute[8], was kindly given to me by Dr. S. Levit. Of the eight cases from the treated wild-type flies, three were lethal in the male, two were point muta-

tions and three were sterile.   One of the lethals was found to be deficient at the locus of scute also.   It was inferred that the reason why so few lethals were found at the locus of yellow was due to the fact that the majority of the breaks took place to the right of the locus of the viability gene, and hence the zygotes were not viable.   The purpose of using the scute[8] stock was to obviate this difficulty.   The left-hand break which produced the scute[8] chromosome took place between the locus of scute and the viability gene, the position of which has thus been changed to some point lying much farther to the right.   This makes it possible to produce deficiencies at the left end without making the zygote inviable.   To produce the lethal mutations at the locus of yellow, gray scute[8] apricot males were x-rayed and crossed to yellow scute miniature garnet forked females.   There were about 4,800 F_1 females examined, and among these were found the 22 yellow females referred to above.   Nine of these were sterile and 13 were fertile, but lethal in the male.

Only two mutations were found at the locus of scute; one of the females was sterile, and the other proved to be lethal in the male.   The very few mutations found at this locus is undoubtedly to be explained on the basis that the eliminated piece carried out the viability gene.

Thirty-four F_1 females showed mutations at the locus of white.   Ten of these flies were sterile, four proved to be point mutations and 20 were lethal in the male.   None of these 20 females had notched wings, showing that the locus of facet was not deficient.   Seven of them had come from treated wild-type flies, showing that the left end with the viability gene had not been eliminated.   The remaining 13 cases were derived from treated Theta flies, and hence the left end, to the right of the locus of white, may have been missing, for the duplication at the right end would allow the zygote to survive.   However, several of these cases were tested to prune, which is not covered

by the duplication, and none of the tested cases was found to be deficient at that locus.

The white-notched flies constitute a group of cases in which the loci of white and facet are both deficient. There were 31 such flies found. Twenty-one of these were fertile and showed, as was to be expected, that they were lethal in the male. The tests showed further that in some of them loci lying to the left or right of the white-notch section were also deficient.

There were 42 females found that had notched wings. Nineteen of these were fertile and lethal in the male. None of these cases was deficient at the locus of white, but one was found to be deficient at the locus of echinus (Table 3, specimen 308).

At the loci of echinus, crossveinless, cut, singed, lozenge and vermilion, the number of mutations found were one, three, three, three, one and five, respectively. The genes for echinus crossveinless and cut were not used extensively as markers, and this explains why so few cases were found at these points. Genes for singed, lozenge and vermilion were used frequently, especially the first two. The very few mutations found at these two loci must be due to the fact that lethals at these points adversely affect the viability of the zygote. The four cases found could not be tested, because a fly homozygous or deficient for either of these genes is sterile.

Of the 53 cases found at the locus of miniature only 17 were fertile, and two of these were point mutations. The 15 lethals were all tested for deficiencies at vermilion on the left and at either dusky or furrowed, or both, on the right, but in no case were any of these loci found to be deficient, that is they did not show pseudo-dominance.

Of the six remaining loci at which mutations were found, those for garnet, forked and carnation deserve consideration. The genes for garnet and forked were used extensively as markers, and they show almost exactly the same number of cases. There were four lethals at garnet and five at forked, and each locus had three

point mutations and one sterile case. At the locus of carnation three cases were found, all lethal in the male. This mutation was found by the writer nearly four years ago, in flies derived from x-rayed larval stages, and although many thousands of flies from treated parents have since been examined, yet a second point mutation at this locus has never been found.

We stated above that it was not possible, from the available data, to compare the rates of mutation as they occur at the several different loci. However, it is possible to compare in a limited way two or more loci with one another, and this can be done irrespective of the number of flies examined. Thus, for example, the mutant genes for miniature, garnet and forked were present as markers in a large number of cultures. If we count the mutations occurring at these loci in all the cultures in which these markers were common, a comparison of frequencies can then be made.

In Table 2 are given all the cases of mutations for six such groups of combinations. In group 1 yellow and white may be compared. There were three mutations

TABLE 2

Groups	1		2		3		4		5			6		
Combinations	y	w	cv	m	sn	m	lz	m	m	g	f	m	f	car
Lethals	1	5	3	7	0	1	0	5	4	2	5	1	0	3
Mutations	1	1	0	0	0	0	0	1	1	0	0	0	0	0
Sterile or no test	1	6	0	12	1	3	0	4	2	0	3	4	0	0
Totals	3	12	3	19	1	4	0	10	7	2	8	5	0	3

found at yellow and twelve at white. This probably does not represent a real difference in mutation rate, because deficiencies produced at the locus of yellow may be due to breaks that resulted in the elimination of a section containing the viability gene, and hence the zygote did not survive. Group 2 shows three mutations at crossveinless as compared to 19 at miniature. Group 3 shows one

at singed and four at miniature. Group 4 had none at lozenge and ten at miniature. Group 5 had seven at miniature, two at garnet and eight at forked. Finally, group 6 had five at miniature, none at forked and three at carnation. If we take the data as a whole, there seems to be no doubt that mutations occur at the locus of miniature more frequently than at any of the other loci, or at least one can say that the F_1 flies with the miniature mutation are more viable.

EXTENT OF THE DEFICIENCIES

The determination of the extent of the deficiencies is a matter of considerable interest. By extent we mean the number of map units that are included in the affected section. Breeding tests show that in the majority of cases only a single known mutant locus is deficient. In all cases, as well as in those of greater magnitude, the deficiency acts as a sex-linked recessive lethal, and prevents the development of the male zygote that inherits the affected chromosome. In Table 3 are given the results obtained in tests made on sixteen cases, which will serve as examples of many similar cases that have been tested. Whenever a mutation at any of the loci was found to be lethal, such cases were further tested for possible deficiencies at the nearest known locus lying to either side, provided stocks with which to make the tests were available. Incidentally, information was secured for several of the more distant loci by using multiple mutant stocks in making the tests.

In the table the minus (—) sign indicates that the particular locus was found to be deficient; those marked with the plus sign (+) indicate loci that were tested and found not to be deficient; the unmarked squares show that tests were not made at these loci; finally the few squares showing a question mark (?) indicate loci that could not be tested because of the nature of the X-chromosome (Theta in all cases).

TABLE 3

Cases	y	sc	br	pn	w	fa	ec	rb	ev	et	sn	lz	ras	v	m	dy	fw	g	pl	sd	r	f	sy	fu	car	bb
	0.0	0.+	0.6	1.0	1.5	3.0	5.5	7.5	13.7	20.0	21.0	27.7	32.8	33.0	36.1	36.2	38.0	44.4	47.9	51.5	54.4	56.5	58.5	59.0	65.5	70.0
247g	?	+	+	+	+	+	+		+	+		+		+	+			+				+				
231c	+	+	+	+	+				+			+		–	+			+				+			+	
244	+	+	+	+	+								+	+								+				
247a	+			+	+				+			+	+	+	–	+	+	+				+				
354	+														+	+		+		+	+	+	+	+	+	
274	+			+	+						+				+	+		+	–	+	+	–	+	+	–	+
268	+			+	+	–	+	+	+		+				+							+		+	+	
262	?	+	+	+	+	–	+	+	+				+	+	+			+				+				
267	?	+	?	+	+	–	+	+	+	+			+	+	+			+				+				
271	?	?	+	+	+	–	+	+	+	+		–	+		+			+				+				
303	+	+	+	+	+	–	+	+	+	+			+		+			+				+				
308	?	+	+	–	+	–	–	+	+			–			+			+				+				
314	+	+	+	–	–	–	–	+	+	+	+		+		+			+				+				
172	?	?	–	–	–	–	–	+	–	+	+			+	+			+				+				
235	?	?	–	–	–	–	–	+	–	+	+		+	+	+			+				+				
231b	?	–	–	–	–	–	–	+	+	+			+	+	+			+				+				

The first seven cases illustrate single locus deficiencies or lethals, as follows: 274g, at white; 231c, at vermilion; 244 and 247a, at miniature; 354, at pleated; 274, at forked, and 268, at carnation. The next four cases show deficiencies at the locus of facet. The female heterozygous for the deficiency has notched wings. However, the character notch is variable, a fact that has been noted by Morgan ('19) and others. The first three cases, 262, 267 and 271, were all obtained by x-raying the Theta X, The duplication attached to the right end of this chromosome can be removed by a crossover, and this makes it possible to test for a deficiency at the locus of broad. It also makes it possible to test the locus of scute, even though the Theta X carries the mutant gene for scute[1]. This was done by the use of one of the scute allelomorphs, known as scute[10], which in combination with scute[1] gives F_1 females that show normal bristle number. If the locus of scute had been deficient, the F_1 females would have revealed scute[10], which they did not. The Theta X carries the mutant gene for yellow also, and consequently it could not be determined whether or not this locus was deficient, because in either event the F_1 fly would show the character yellow. Case 271 could not be tested at the loci of scute and broad in this way, because of the presence of a non-crossover factor, due perhaps to an inversion.

The five remaining cases all showed deficiencies at two or more loci that lay adjacent. Numbers 308 and 314 were derived from treated wild-type flies, while the other three, nos. 172, 235 and 231b, came from the treated Theta X-chromosomes. The deficiency in 308 was found to include the loci of facet and echinus, while that of 314 includes the loci of white, facet and echinus. The deficient section in the last case therefore extends over a distance of at least four map units.

The three cases obtained from the Theta X have deficiencies that are still more extensive. Thus 172 and 235 were both found to be deficient at prune, white facet

and echinus, which represents a map distance of at least four and a half units. It was suspected that in each of these two cases the entire left end, from yellow to echinus, was deficient, but it was not possible to test any one of the three loci of yellow, scute or broad, because of the presence of the Theta fragment on the right end. If the fragment is crossed out, the female receiving the crossover chromosome with the deficiency is not viable, because of the absence of the viability gene. If the deficiency is due to the loss of a piece of the chromosome, it might be possible to establish the fact by making cytological studies. My colleague, Dr. T. S. Painter, made cytological studies on about a dozen cases of deficiencies, but in no case was he able to obtain positive evidence that a piece was missing, even in such cases as 172 and 235.

We now know why it is difficult, if not impossible, to detect by cytological observations a missing piece from the left end of the X-chromosome, even though such a piece may include six or seven map units. The fact is such a piece is too small physically to permit of a detection of its absence from the remainder of the chromosome. This fact was established for the first time by a study of the mottled-notched case, No. 231b, which is here included for the purpose of showing that the deficiency phenomenon is due, at least in this case, to an actual loss of a piece of the chromosome.

The mottled-notched case was very similar to cases 172 and 235, but differed from them in that the broken-off piece became translocated to a fourth chromosome. The translocation was unstable, and the occasional loss of the piece from the fourth during somatogenesis resulted in producing mottled eyes and variable notched wings (Patterson, '32). It was found that the translocated piece was only about three times the size of the dot-like fourth, with which it could be compared (Patterson and Painter, '31). This shows why it is practically impossible to detect the absence of such a small piece from the affected chromosome.

DISCUSSION AND CONCLUSION

We have shown that a very large number of the muta-
tions induced by x-radiation at known loci are lethal in
effect, even though the measurable limits of the deficiency
may not exceed that of the locus of the mutant gene.   A
lethal mutation of this character has been termed a gene
deficiency, in contrast to a sectional-deficiency caused by
the loss or inactivation of a definite section of the chro-
mosome and in contrast to a chromosome-deficiency
caused by the loss of an entire chromosome through non-
disjunction ("Genetics of Drosophila," 1925).

If, as has been shown, the loss of an entire chromosome,
or of half of a chromosome, or of a still smaller but
definite measurable section (case 231b), all produce the
deficiency phenomena, then at what point in the descend-
ing series must one cease to regard the deficiency as due
to a loss and assign to it some other cause?   These so-
called gene-deficiencies not only are lethal for the male,
but they also show pseudo-dominance, both characteristic
of sectional-deficiencies.   It is also stated that they ex-
hibit "exaggeration" of the mutant character (Mohr,
'23).   In these respects then the single locus lethal mu-
tations are similar to the more extensive deficiencies,
such as found in case 231b.   It seems logical to assume
that deficiencies such as were observed in cases 314 and
308 are due to losses of definite sections of the chromo-
some.   This, however, is difficult to prove, and it becomes
more difficult still to prove that the single locus lethal is
due to a loss of a mutant gene, even though this might
seem to be the next most logical assumption to make.

Various suggestions have been made in order to ex-
plain the underlying causes of deficiencies, especially by
Bridges and Mohr.   The second author has considered
most of these in his papers dealing with his Deficiency-
Notched 8 (Mohr, '19, '23).   He first suggested in 1919
that this case could be explained best on the basis of the
"chain mutation" theory, that is, that the deficiency phe-

nomena were due to a change brought about through a simultaneous mutation of an entire chain of adjacent genes. Later (1923) he expressed doubts as to the correctness of the chain mutation theory, and was inclined to support the loss hypothesis. He believed that he was able to detect a slight difference in size of the central ends of the X-chromosomes of the Notch 8 females, but it was later shown that the central end of the X-chromosome, as it appears in the equatorial plate, represents the fiber-bearing end and not the one near which his notched-deficiency occurred.

With reference to the single locus lethals, it may be that we are dealing with cases that are due to causes differing from those that underlie the more extensive deficiencies. It is conceivable that such mutations could arise in any one of the following ways: (1) By a recessive mutation having a lethal effect, but not including any adjacent genes; (2) by the loss of the mutant gene together with one or more adjacent genes that are essential to the life of the fly (Bridges); (3) by a recessive mutation at a known locus and a lethal mutation in a near-by gene (Mohr); (4) by a recessive mutation at a known locus and a lethal mutation in a gene situated at some distance from the mutant gene. In case the mutant gene and the lethal gene are identical, or lie so close to each other that crossing-over can not take place between them, it would be impossible to use the crossover method for the purpose of locating the lethal gene. In this connection it is of interest to point out that reverse mutations are usually not associated with lethal effects. Thus, Muller and the writer obtained five reverse mutations in F_1 females at the locus of forked, and none of these was lethal (Patterson and Muller, '30). It is true that the number of cases was small, yet in a corresponding number of direct mutations, at least four out of the five would have been lethals.

There is described in the paper an effective method for obtaining lethal mutations of the so-called deficiency type

at any known mutant locus. The method consists in x-raying the wild-type fly (or the Theta fly) and then crossing the treated individual to an untreated fly that has an X-chromosome ''marked'' with several recessive mutant genes. It was found that seven out of every eight mutations occurring in the X-chromosome represented lethal mutations.

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RECURRENCE OF A PECULIAR GENETIC RECOMBINATION IN THE SPIKE DENSITY OF WHEAT[1]

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Two earlier papers (5, 6) have reported the occurrence of a partially unexplainable transgressive segregation in the spike density of crosses involving Federation×Sevier and Kanred × Sevier wheats. Recently, a cross made between Ridit and Sevier has brought a recurrence of the same peculiarity, whereby a true club wheat· is obtained from a cross of lax with an intermediate form in a ratio which seems to require only a single major factor difference for explanation. Even more peculiar is the non-recovery of one of the parents and the rare recovery of the other.

EXPERIMENTAL PROCEDURE

The cross between a pure line of Sevier (Sevier 59) and Ridit was made at the Utah Station in 1927. The F_1 plants were grown in 1928 and the F_2 families in 1929. The genetic study herein reported was made on F_3 progenies, the kernels from each of the 299 F_2 plants being used to seed an F_3 row. This made it possible to use the breeding behavior of the F_3 progenies as a basis for the genetic classification of the F_2 plants. This method has proved to be definitely superior to the method of classifying the F_2 material directly, since so many characters exhibit such an intermediate appearance in the F_2 that it is impossible to classify all the F_2 plants correctly.

The F_3 progeny rows were spaced 1 foot apart with a space between kernels of 2 to 3 inches. After each tenth

row the two parental varieties, Sevier 59 and Ridit, were sown side by side.   There were, equally spaced throughout the breeding plat, 29 rows of each parental variety. Data were taken on these parent rows in the same manner and at the same time as on the progeny rows.

In 1930, by use of the F_3 families, the inheritance of awnedness, glume color and kernel color was determined by observation.   A leading spike from each plant was measured to determine the length of ten central rachis internodes.   A few rows contained less than 40 plants; most of them contained more, in which case the data from only the first 40 chosen at random were recorded.   All, however, were examined for kernel color and other characters determined by observation in order to be sure of obtaining representative ratios.

No theory of inheritance was considered until all data were recorded and tabulated.   Although the data taken on the F_2 plants were not used in genetic classification, they proved valuable in two respects: In the first place, the time required for checking the F_3 rows in the field was reduced about 50 per cent. by using these data; and secondly, comparison of the F_2 plant with the F_3 row minimized the possibility of error.

Parental Material

Sevier 59: Sevier 59 is a selection from the variety Sevier which was discovered and named by Stewart in Sevier County, Utah, in 1918.   The spike is short, fully awned, and compact but not clubbed.   The glumes are glabrous, bronze in color and rather stiff.   The kernels are white, hard and translucent.   The straw is short to mid-tall and weak, making it easy to lodge.   The variety is commercially important in Sevier Valley, Utah, where it gives good yields and exhibits some resistance to the physiological forms of black stem rust that occur there.

Ridit: Ridit was developed at the Washington Agricultural Experiment Station from a cross between Turkey

and Florence. The head is lax with white, glabrous chaff, and short apical awns. The straw is stiff and the kernels are red, hard and vitreous with good milling quality. The outstanding characteristic of this wheat is its resistance to both types and several physiological races of bunt smut.

A summary of the contrasting parental characters is presented in Table 1.

TABLE 1

SUMMARY OF THE CONTRASTING PARENT CHARACTERS

Character	Ridit	Sevier 59
Glume color	White	Bronze
Kernel color	Red	White
Awnedness	Short apical awns	Fully awned
Spike density	Lax (10 intern. about 45 mm)	Mid-dense (10 intern. about 34 mm)
Smut resistance	Highly resistant	Susceptible
Stiffness of straw	Rather stiff	Extremely weak

Typical parent spikes and a head taken from the F₁ generation are shown in Fig. 1.

FIG. 1. Spikes of the two parents, Ridit and Sevier 59, and of the F₁ generation. The spike density of the F₁ is almost identical with Sevier 59, the more dense parent.

EXPERIMENTAL RESULTS AND THEIR INTERPRETATION

The breeding behavior of every F_3 row was summarized and placed in a table for use in this study.

The character of spike density involved measurements for about 40 plants in each F_3 progeny row. From these measurements, means, length of 10 rachis internodes, standard deviations and coefficients of variability were calculated. Comparisons of these biometrical constants made it possible to segregate the progenies into two homozygous and one heterozygous group for spike density. In the biometrical studies the mean value of the plants in each F_3 row was used, this being considered more accurate than a single figure from one F_2 plant.

In the present study the F_1 resembled rather closely the Sevier parent in density. In the F_2 segregation several degrees of spike density were observed, some plants having more lax spikes than Ridit and others having much denser ones than Sevier 59.

The parental rows of Ridit had an average spike density of 44.58 mm and showed coefficients of variability ranging from 3.8 per cent. to 6 per cent., with a mean of 4.73 per cent. The parental rows of Sevier 59 had an average spike density of 33.9 mm, and the coefficients of variability ranged from 4.4 per cent. to 8.7 per cent., with a mean C.V. of 5.78 per cent.

The mean length of ten internodes of all the homozygous dense rows was 23.6 mm, and the coefficients of variability in this group ranged from 5.4 per cent. to 16.2 per cent., the mean C.V. being 10.5 per cent. The homozygous lax progenies averaged 52.83 mm for length of ten internodes and showed a mean C.V. of 6.67 per cent. The heterozygous rows showed coefficients of variability ranging from 22 per cent. to 40.8 per cent., with a mean of 30.7 per cent. Only one of the heterozygous progenies had a C.V. as low as 22 per cent., and few were below 27 per cent.

This method of spike density classification showed the three segregating groups in a clear-cut manner. The

TABLE 2

MEAN SPIKE DENSITY AND COEFFICIENT OF VARIABILITY (C.V.) CLASSES OF RIDIT AND SEVIER 59 WHEATS, AND OF THE THREE GROUPS OF F_3 PROGENIES OF THEIR CROSS—HOMOZYGOUS DENSE, HETEROZYGOUS, AND HOMOZYGOUS LAX.

Parent or progeny	Spike density classes															Total	C.V. classes
	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60		
Ridit Parent									1	5						6	3
									3	20						23	6
Totals									4	25						29	
Mean C.V.																	4.73
Average spike density																	44.5
Sevier 59 Parent						3										3	3
					3	21										24	6
						2										2	9
Totals					3	26										29	
Mean C.V.																	5.78
Average spike density																	33.9
Homozygous Dense Progeny	3	5	4	2												14	6
	2	12	20	4												38	9
		3	11													14	12
		1	2													3	15
Totals	5	21	37	6												69	
Mean C.V.																	10.5
Average length of 10 rachis internodes																	23.6

TABLE 2—Continued

Parent or progeny	Spike density classes															Total	C.V. classes
	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60		
Heterozygous Progeny						1										1	21
				1	1	1	2	3	2	1						11	24
					3	10	17	7	3							41	27
				2	6	18	20	7	1							54	30
				1	10	15	12	2		1						40	33
				1	5	5	1									12	36
					3	2										5	39
Totals				5	29	52	52	19	6	2						164	
Mean C.V.																	30.7
Average spike density																	34.75
Homozygous Lax Progeny											1		1			2	3
										1	11	16	17	5	1	51	6
											5	3	2	3		13	9
Totals										1	17	19	20	8	1	66	
Mean C.V.																	6.67
Average spike density																	52.83

The vertical columns represent spike density classes of the means of the parental rows and F₃ progenies, groups horizontally into three major progeny groups and the two parental groups. The horizontal lines within each group represent the coefficient of variability classes.

mean spike density classes and the coefficient of varia-
bility (C.V.) classes of the parent rows and of the three
segregating groups are shown in Table 2.

In Fig. 2 the spike density curves of the Ridit and
Sevier parents, together with the curves for the three
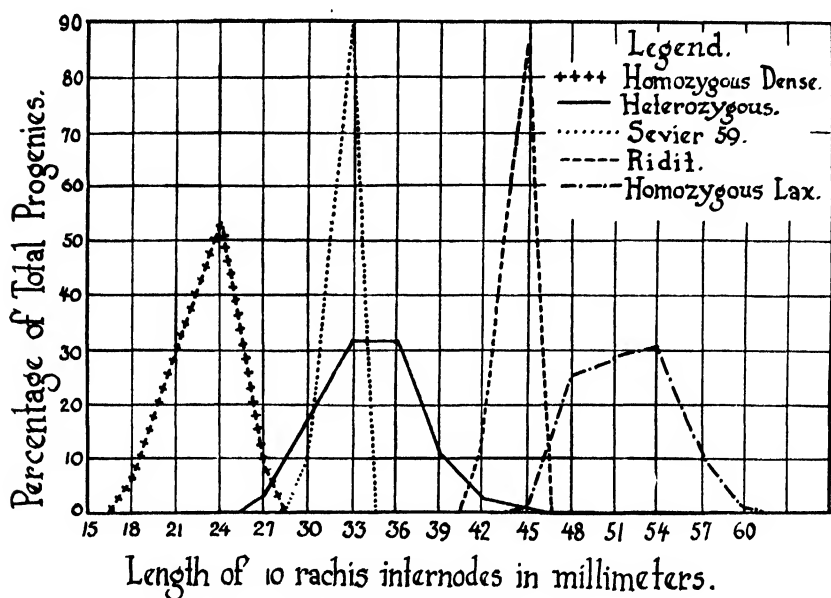groups of F_3 progenies, are shown graphically.

FIG. 2.   Spike density curves of Ridit and Sevier 59, parents, and of three
progeny groups: Homozygous dense, Heterozygous, and
Homozygous lax.

Fig. 3 shows the rachis and spikes from parents and
progeny of the Ridit $\times$ Sevier 59 cross.

The mean coefficients of variability were greater for
each of the two true-breeding F_3 progeny groups than
for either of the two parents.   In comparing the varia-
bility of the homozygous dense with the homozygous lax
it was found that the homozygous lax group showed
much less variability than the homozygous dense rows;
it was also shown that Ridit was less variable than
Sevier 59, possibly due to the greater mean length.   The
coefficient of variability of the least variable hetero-
zygous progeny exceeds that of the most variable homo-
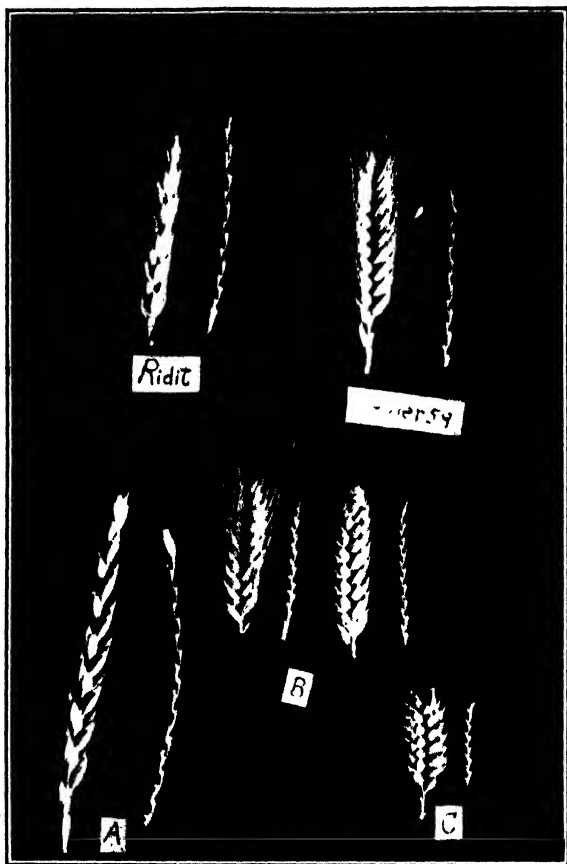zygous progeny in every case.

FIG. 3.   Spikes and rachises of the two parents, Ridit and Sevier 59, and of the three progeny groups: *A*—homozygous lax, *B*—heterozygous, and *C*—homozygous dense.   Inspection reveals that the lax progenies are more lax than Ridit and the dense progenies more dense than Sevier 59.

When counts were made of the F_3 progenies in each of the three groups, it was found that there were 66 homozygous for lax spikes, 164 heterozygous and 69 homozygous for dense spikes.   Table 3 gives the calculation for closeness of fit on a one-factor difference for spike density.

This value of P (.242) indicates that the probability is very great that the proposed one-factor hypothesis is essentially correct.

There are several considerations, however, which lend to the belief that there are also minor factors involved in

TABLE 3

CLOSENESS OF FIT OF THREE GROUPS OF F_3 FAMILIES COMPARED TO A 1:2:1 RATIO

Spike density group	Observed value (O)	Calculated value (C)	O – C	$(O-C)^2$	$\dfrac{(O-C)^2}{2}$
Homozygous dense	69	74.75	5.75	33.06	.442
Heterozygous	164	149.50	14.5	210.25	1.407
Homozygous lax	66	74.75	8.75	76.56	1.024

$$X^2 = 2.873 \qquad P = .242$$

the expression of spike density in this cross. In the first place, the homozygous dense progeny were without exception more dense than the dense parent. That this was a real difference in the length of ten internodes was 9.2 times the probable error. This is shown in Table 4 where the more dense parent (Sevier 59) is compared with the dense homozygous progenies and the more lax parent (Ridit) is compared with the lax progenies.

TABLE 4

MEAN SPIKE DENSITY OF PARENTS COMPARED WITH HOMOZYGOUS SEGREGATION GROUPS TO SHOW TRANSGRESSIVE SEGREGATION

	No. of rows	Mean length of 10 internodes	Difference/P.E.
Sevier 59	29	33.9 ± 0.73	
Homozygous dense progenies	69	23.6 ± 0.85	9.2
Difference		10.3 ± 1.12	
Ridit	39	$44.56 \pm .602$	
Homozygous lax progenies	66	$52.83 \pm .544$	7.7
Difference		$8.25 \pm .107$	

The odds are more than a billion to 1 that there is a significant difference. The lax group also showed a far greater degree of laxness than the lax parent (Ridit). The odds are more than 500,000 to 1 that there is a real difference.

The spike density of Sevier was not recovered at all in the F_3 and that of Ridit only in one case. Since there

were 299 F₃ families, the number of segregates breeding true to either one of the parents is so small that it is clear that some minor factors are involved. This is further borne out by a comparison of the spike density ranges of the parents and F₃ progeny. A summary of the range of densities is given in Table 5.

TABLE 5

The Range of Mean Spike Densities and the Mean of Mean Spike Densities of Ridit and Sevier 59 and of Three Groups of F₃ Families. Also Coefficients of Variability (C. V.) of Mean Spine Densities for All Groups

Parent or segregate	Range of spike densities	Mean spike density	C. V. of mean spike density
Ridit	42.2 – 46	44.53 mm.	4.73
Sevier 59	31.7 – 34.3	33.9 mm.	5.78
Homozygous dense	19.2 – 28.3	23.6 mm.	10.5
Heterozygous	26.7 – 46.7	34.75 mm.	30.7
Homozygous lax	46.6 – 58.6	52.83 mm.	6.67

On an average, the variability of the means of homozygous F₃ families is about twice as great as that of the 29 parent rows. This, added to the fact that there is a series of steps throughout the range of variation, points to one or more minor factors which modify the expression of the one major factor for spike density.

As yet no genetic hypothesis has been advanced that will explain the fact of wide transgressive segregation in both directions and still account for the infrequent recovery of the spike density of the parents.

Soil Heterogeneity

A study was made of the soil heterogeneity of the experimental plat. The Harris (2) method was used in this study and as the 29 pairs of parent rows were grown side by side at regular intervals throughout the plat, they were used as the basis.

The contiguous rows received identical treatment and the extent of fluctuation due to environment was deter-

mined by a correlation study between the spike density measurements of the Ridit and the Sevier 59 parents. According to Harris, this correlation coefficient measures the degree to which nearby plats or rows are different and allows a comparison of soil heterogeneity. The coefficient will be in proportion to the soil heterogeneity, that is, the larger the positive value of r the greater the degree of soil heterogeneity.

In this case the coefficient of correlation was $+.29 \pm .11$. When the value of r is changed to per cent. by the formula $V = 100 \left(1 - \sqrt{\frac{r^2}{1}}\right)$, (V = variation in per cent.), it is found that there is 5 per cent. variability. This indicates that only a small part of the variation in this experiment was due to soil heterogeneity. Such small environmental influence on the character studied adds much to the validity of the conclusion that spike density segregation was transgressive and otherwise peculiar.

Each F_3 plant was classified by inspection for glume color and the breeding behavior of the rows was thus determined. Of the 299 progeny rows, 77 bred true for bronze chaff; 75 bred true for white chaff; and 147 segregated. These numbers suggest a $1:2:1$ ratio or a one-factor difference for color of glumes. A comparison of this hypothesis with the actual count is made in Table 6.

TABLE 6

GOODNESS OF FIT OF THREE GROUPS OF F_3 PROGENIES FOR GLUME COLOR COMPARED TO A $1:2:1$ RATIO

Progeny group	Observed value (O)	Calculated value (C)	O – C	$(O-C)^2$	$\frac{(O-C)^2}{2}$
Homozygous bronze	77	74.75	– 2.25	5.06	.067
Heterozygous .	147	149.50	+ 2.50	6.25	.041
Homozygous white	75	74.75	– .25	.06	.003

$X^2 = .111$ $P = .9256$

This value of P (0.93) indicates that a worse fit might be expected in 93 cases out of 100, due to chance alone.

There seems little doubt regarding there being a one-factor difference for glume color.

The F_1 plants had lighter colored glumes than Sevier and were darker than Ridit; also, in the F_2 and F_3 generations there were degrees of bronzeness which varied from the dark bronze color of Sevier to shades so light as to be almost indistinguishable from plants classed as white. These two facts suggest an incomplete dominance of the factors for bronzeness, although the ratios seem to prove the dominance of the bronze color.

Kernel Color

In the cross here reported, red kernel color proved dominant in the F_1. Twenty-one of the 299 progenies studied in this cross produced white kernels in the F_2. This is 7 per cent. of the total. The remaining 278 plants, or 93 per cent., produced red kernels. Minute examination of red kernels from a large number of plants disclosed no significant difference in the degree of redness, although Hayes and Garber (3) report that the factors for kernel color are often cumulative and that two factors will give a darker color than will one of the factors alone.

The percentages of red and white kernels suggested a two-factor difference for this character. The breeding behavior in the F_3 established this hypothesis. All the white-kerneled plants bred true in the F_3, while the remaining 278 showed the following breeding behavior:

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141 plants bred true for red kernels
 67 segregated in the ratio of  3 red: 1 white
 70 segregated in the ratio of 15 red: 1 white
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On the basis of a two-factor difference for kernel color it was calculated that each 16 plants should breed as follows:

Breeding true for red grain	7
Segregating 15 red: 1 white	4
Segregating 3 red: 1 white	4
Breeding true for white grain	1
Total	16

When this theoretical expectation was compared to the actual data, a fairly close fit was observed. The group breeding true for red grain numbered about 7 per cent. more than was calculated, while the two segregating groups were each a little smaller than was expected. The true-breeding white-kerneled plants approached extremely close to expectations. Seven of the segregating groups were on the border between 3:1 ratios and 15:1 ratios; in the final grouping of data (given above) it was decided, therefore, to put three of these groups in the 3:1 class and the other four in the 15:1 class. If any of the ratios were seriously affected by these seven doubtful progenies, an F_4 could be grown and its breeding behavior would eliminate the doubt in this classification. Outside of these seven groups, no difficulty was experienced in classification of kernel color as all of the other 299 progenies presented clear-cut breeding behavior. The two-factor hypothesis suggested to explain this breeding behavior was made the basis of the closeness of fit comparison in Table 7.

TABLE 7

CLOSENESS OF FIT OF FOUR GROUPS OF F_3 PROGENIES ON A TWO FACTOR DIFFERENCE IN THE PARENTS FOR KERNEL COLOR, COMPARED WITH A 7:4:4:1 RATIO

Progeny group	Observed value (O)	Calculated value (C)	O − C	(O − C)²	$\frac{(O-C)^2}{2}$
Homozygous red grain	141	131	10	100	.76
Segregating 3 red: 1 white	67	74.7	− 7.7	58.4	.77
Segregating 15 red: 1 white	70	74.7	− 4.7	22.09	.294
Homozygous white grain	21	18.6	2.4	5.76	.306

$$X = 2.13 \qquad P = .5687$$

AWNEDNESS

The awns were classified according to appearance into three groups, which were known merely as awns 2, 3 or 4. Those plants in the awn 2 class bore short beaks along

the side of the spike with partial awn development at its apical part.   These plants resembled the Ridit parent. Those in awn class 4 were fully awned and bore awns corresponding to those on the Sevier parent.   The plants in awn class 3 were intermediate in their awn development, were considerably longer than those of class 2, and short awns were found as far as half way down the spike. In this case the apical awns were considerably longer than those of awn class 2 and generally rather long awns could be found over all the apical half of the spike.   It resembled the awn development on the F_1 plants.
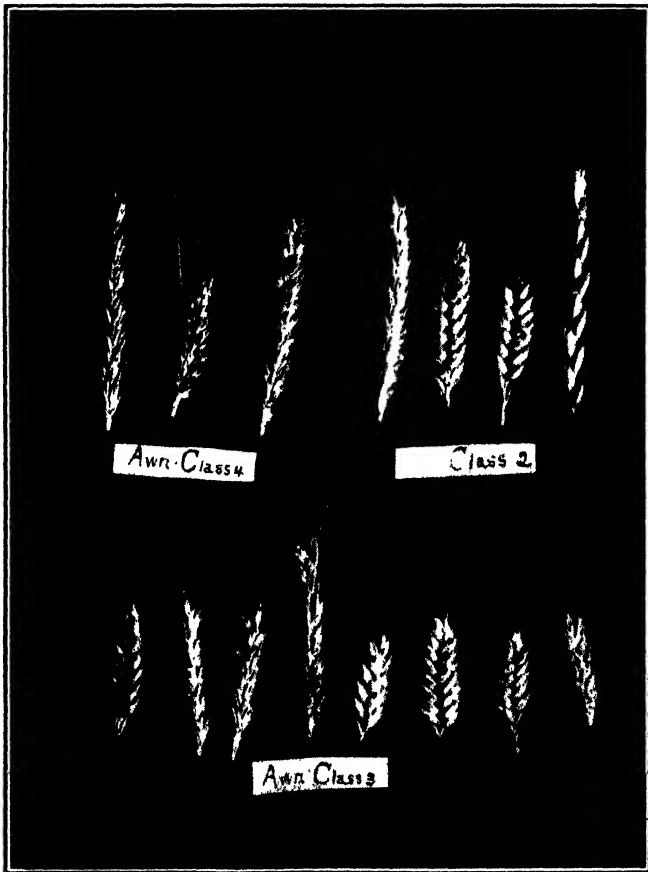

Fig. 4.   The three awn class groups: Awn class 4, homozygous fully awned similar to the Sevier parent; awn class 2, homozygous awn-tipped similar to Ridit; and awn class 2 which is heterozygous.

The awn types of the parents and also of the F_1 are shown in Fig. 4. Fig. 2 shows several plants of each of the three awn classes recovered in the F_3 generation.

All the F_2 plants which were classed as having awns 4 bred true in F_3, but it was impossible in the F_2 to distinguish between the plants which had strong awns 2 and those which had awns 3. The F_3, however, clearly separated these two groups. In taking the data on the F_3 progenies, clear-cut cases of segregation could be seen, and these groups were easily classified.

When the 299 F_3 progenies were studied as a basis for the genotypic classification of the F_2 plants, it was found that 68 rows were breeding true for awn class 4; 150 rows were segregating for all three awn classes. There was great variation in the awn types of these segregating rows. The remaining 81 rows bred true for awns class 2. This classification approaches a $1:2:1$ ratio and suggests a one-factor difference.

The goodness of fit between the observed data and the calculated frequencies is presented in Table 8.

TABLE 8

Goodness of Fit of 3 Awn Genotype Classes of the F_2 Progenies When Compared with 1: 2: 1 Ratio Which Would Theoretically be Obtained in the Segregation of a One Factor Difference

Progeny group	Observed value (O)	Calculated value (C)	O − C	(O − C)²	$\dfrac{(O-C)^2}{2}$
Homozygous awns 4	68	74.75	− 6.75	45.56	.6100
Heterozygous	150	149.50	.50	.25	.0016
Homozygous awns 2	81	74.75	6.25	39.06	.5225

$$X^2 = 1.134 \qquad P = .5755$$

On the basis of 3 awn classes, $X^2 = 1.134$ and P is .5755, a rather close fit; in fact a worse fit would be expected in 58 out of 100 cases, due to chance alone.

Summary

Spike density exhibited peculiar inheritance. Three groups were found in the F_3 progenies in numbers which

suggested a 1:2:1 ratio, with a goodness of fit as indicated by $P = .242$. The homozygous dense progenies transgressed the range of the dense parent in mean spike density and the homozygous lax progenies transgressed the range of the lax parent, by odds of millions to 1 that the transgression is significant. The dense parent is not recovered in a single progeny and the lax parent in very few. The range of densities of the heterozygous progenies covers completely the range of the denser parent. There seem to be one or more minor factors present in the inheritance of spike density.

A one-factor difference, suggested as the basis for glume color inheritance, gave, when compared to a 1:2:1 ratio, a P of .93.

Kernel color was inherited on the basis of a two-factor difference. When the breeding behavior in F_3 was compared to that expected from a two-factor difference, a fairly close fit (of $P = .57$) was obtained. Close scrutiny of the red kernels failed to reveal any cumulative effect of the factors for red grain.

Awnedness was found to be inherited on the basis of a single-factor difference. The numbers expected on the basis of a 1:2:1 ratio fit the observed numbers closely, as indicated by $P = .58$.

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HOMOGAMY IN THE TOAD

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HOMOGAMY is of some importance for the theory of evolution; for it is one of three necessary conditions for species differentiation, the others being variation and inheritance. That is, if differentiation is to occur, deviates must first arise; they must mate with similar deviates; and they must transmit the deviation to their descendants. In the absence of the second step (homogamy) the deviation must soon disappear by averaging back into the population. The degree of homogamy is of consequence only in connection with time; a homogamy (correlation) coefficient of .1, if real, is of as much theoretical import as one of .9.

The first quantitative study of homogamy was Pearson's result on eye color in man, published in 1900 (19); it yielded a coefficient of $.26 \pm .03$.[1] In 1903 appeared Pearson and Lee's coefficients of $.28 \pm .02$ for stature, $.20 \pm .02$ for span, and $.20 \pm .02$ for left forearm (20); Boas' coefficient of $.15 \pm .10$ for cephalic index (2); and a cooperative study presumably inspired by Pearson yielding coefficients for longevity in three English populations of $.22 \pm .02$, $.25 \pm .02$, and $.20 \pm .02$ (1). Lutz, in 1905, determined the coefficient for age as $.76 \pm .006$ (16). Pope, Goring and Elderton, in 1908 and 1909 (6, 10, 22), found coefficients for tubercular infection of $.32 \pm .003$, $.30 \pm .04$, $.16 \pm .03$, and $.01 \pm .025$, with some evidence that the homogamy is greater in the superior social groups; in addition, Goring found a coefficient for freedom from constitutional disease of $.11 \pm .03$, and Elderton one for general health of .27 (probably $\pm .04$). Harris, in 1912, recalculated data due to Galton and arrived at a contingency coefficient of .34 for hair color (11).

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[1] Contingency; $.10 \pm .04$ by product-moment method.

Williams, Bell and Pearson, in 1914 (26), found a coefficient of .14 ± .016. Willoughby (unpublished data) has found coefficients for number of siblings of from .36 for parents of college students to traces in eighteenth-century populations. The average of these coefficients for physical characters is .24.

For behavior characteristics, seventeen coefficients of contingency and tetrachoric coefficients of correlation, derived from rated attributes by Woods (28), Elderton (6), Schuster and Elderton (23), and Goring (10), average .27. This value is probably spuriously low, due to the unreliability of ratings, for Burks (3) Freeman *et al.* (8), Jones (14) and Willoughby (27), publishing in 1928, derived the values .44, .47, .57, .61 and .62 for ability as measured by standardized intelligence tests. These, however, are characteristics of a different order from the physical measures heretofore considered. Jones (13) has usefully summarized the literature on homogamy in man up to 1929.

The first quantitative observation on homogamy in infra-human animals was that by Tower in 1906 (25), on the potato beetle *Leptinotarsa;* the measurement used was apparently elytron length, and the animals seem to have been grouped into size classes instead of measured; further, the results are reported in terms of the percentage of each size class of males mating with each size class of females. They have been recalculated by the present writers on the basis of reasonable assumptions, and yield a product-moment coefficient of .89 ± .01; Tower infers, we believe wrongly, that the population is therefore tending constantly toward mediocrity. In the same year Kellogg (15) published information on the number of spots on pairs of the lady-bird beetle *Hippodamia,* from which he concluded without analysis that there was no homogamy; Pearl, in a bibliography annotation (18), also without analysis, remarked that this conclusion was in direct contradiction to the facts pre-

sented. On the somewhat doubtful assumption that the number of spots may be taken as a continuous variate in the statistical sense, and using a symmetrical table to allow for the fact that the data reported do not distinguish the sexes, the coefficient works out at .32 ± .08. Pearl, in 1907 (17), published the results of an exhaustive study of *Paramecium,* giving coefficients averaging .61 for length, .30 for breadth, and .43 for index (breadth/length), with cross-coefficients averaging .11 (all ± about .04) ; and these results were confirmed by Jennings (12) in 1911, with coefficients for length averaging .38 ± .04 for wild cultures, .94 ± .01 for cultures containing two species of different sizes, .25 ± .05 for pure races, and for breadth (wild cultures) averaging .33 ± .03.   Enriques (7), working with *Chilodon uncinatus,* found a zero coefficient for the early stages of conjugation and one of .40 for the later stages. Crozier (4) working in 1918 with *Chromodoris zebra,* a nudibranchiate gastropod, found a correlation for total length of .61 ± .02, one of .52 ± .05 for mantle length, one of .14 ± .08 for volume, using pairs which had copulated in the field; and a total length correlation of .72 ± .02 for pairs formed in the laboratory (original matings) ; repetition of the latter experiment with specimens[2] collected eight months later gave a correlation of .97 ± .01. Crozier and Snyder (5) found for length in the amphipod *Gammarus locusta* a coefficient of .91 ± .01, and for the related species *Dikerogammarus fasciatus* .69 ± .04 (the former coefficient was reported in a preliminary paper, evidently on the same material (24), as .80 ± .03). The present investigation is the next in temporal order, and represents the first investigation in an infra-human vertebrate. Valuable theoretical material may be found in a paper by Wright (29), as well as in several of the references cited for factual findings.

The material of the present study comprises 86 pairs of *Bufo americanus,* captured in amplexus in the various

[2] Nineteen pairs.

breeding pools near Clark University during the two weeks beginning April 22, 1931. They were transported to the laboratory (where the temperature was a little higher than in the field), retained until it was convenient to measure them, measured and "banded" with linen thread and beads, retained a few more days (perhaps an average of a week in all), and liberated. Most of the eggs were extruded during retention in the laboratory, and frequent remating occurred; in all but a few cases the homogamy measured is that between the pairs formed in the field, but there is of course no guarantee that this was the first mating of the season. There were fewer rematings in a clean tank than in one with soil and vegetation. Eight pairs were lost by separating before being banded or measured, or by losing the bands; the measurements of the still banded member were used in the self-correlations.

Four measurements were taken on each animal: jaw width at the widest point (J); length of the extended left leg from dorsal margin of the cloacal orifice to tip of middle toe (L); distance between the lateral margins of the iliac crests ("sacral humps" of some writers) (S); and distance from tip of urostyle to anterior margin of left iliac crest (U). In case of obvious pathology in the left leg, the right was measured and the result used indiscriminately in the computations.[3] All measurements were made by the same person (C. M. P.)[4] with a mechanic's outside caliper with rounded points, and read

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[3] There were four females and one male with broken left toes, and one male with seven toes on the left foot; one female had a neoplasm in the throat region; one female had an injury to the left side of the pelvis, and one male had a fragment (evidently resorbed) broken from the urostyle, so that the remainder, 17 mm long, ended in a sharp point; three males had the left eye hemorrhagic, and of these one was unable to open the nictitating membrane; one female had a right eye very much reduced in size, and one male had no left iris.

[4] For indispensable assistance in the measuring, under trying conditions, indebtedness is gratefully acknowledged to Messrs. David Potter, George McCabe and William Snape.

against a celluloid millimeter scale fixed to the table (due precautions being taken to avoid parallax error, and most of the readings being checked by a second reader); the leg measurement was made with the scale directly. The measurements were recorded in millimeters, and the estimations were made to the nearest .5 mm.

The distributions of direct measures are as follows (index is lower limit of class throughout):

J	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	N	M	σ	V
m		1	9	17	25	12	16	11		1						92	22.66	1.65	7.3
f		1	1	3	5	9	9	15	12	9	12	6	2	3	1	88	26.81	2.68	10.0

$$d_M/\sigma_d = 12.4 \qquad d_\sigma/\sigma_d = 4.4$$

L	66	70	74	78	82	86	90	94	98	102	106	110	114	N	M	σ	V
m		2	11	12	27	15	12	7	5		1			92	81.42	7.39	9.1
f			2	5	9	13	19	13	16	6	2	1	1	1	88	89.07	8.25	9.2

$$d_M/\sigma_d = 20.6 \qquad d_\sigma/\sigma_d = 3.26$$

S	12	13	14	15	16	17	18	19	20	21	22	23	24	N	M	σ	V
m		1	9	33	19	18	9	2	1					92	15.11	1.31	8.7
f	1		5	7	12	15	20	14	8	3	1	1	1	88	17.84	1.94	10.9

$$d_M/\sigma_d = 11.1 \qquad d_\sigma/\sigma_d = 3.6$$

U	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	37	N	M	σ	V
m	1	2	9	19	15	12	13	10	6	3	1							91	22.91	2.08	9.1
f	1			1	4	2	9	10	16	13	11	6	8	4	1	1	1	88	26.88	2.74	10.2

$$d_M/\sigma_d = 10.9 \qquad d_\sigma/\sigma_d = 2.5$$

It is evident that the females are significantly larger and more variable than the males. Occasional sex differences also appear in the morphological indices formed by dividing each measure by the others (decimal points have been omitted):

J/L	245	250	255	260	265	270	275	280	285	290	295	300	305	310	315	320	325	335	N	M	σ	V
m	3	3	1	8	9	8	16	9	12	9	8	3		2	1				92	280	15	5.2
f				4	2	4	1	6	8	11	17	11	10	5	4	3	2	88	301	15	5.1

$$d_M/\sigma_d = 9.4 \qquad d_\sigma/\sigma_d = 0$$

J/S	110	115	120	125	130	135	140	145	150	155	160	165	170	175	180	N	M	σ	V
m		1	1		1	3	15	14	30	16	7	2	1	1		92	151	8	5.5
f			1	6	12	18	29	8	8	1	3		1			87	151	9	5.7

$$d_M/\sigma_d = 0 \qquad d_\sigma/\sigma_d = 2.3$$

J/U	82	86	90	94	98	102	106	110	114	118	122	N	M	σ	V
m		2	5	11	15	18	22	13	2	3			91	100	32	32
f		1	3	8	21	18	20	12	3		1	1	88	100	32	32

$$d_M/\sigma_d = 0 \qquad d_\sigma/\sigma_d = 0$$

L/S	410	430	450	470	490	510	530	550	570	590	610	N	M	σ	V
m	1			4	5	27	26	15	9	4	1	92	540	31	5.7
f		3	7	16	34	15	9	4				88	500	27	5.4

$$d_M/\sigma_d = 9.3 \qquad d_\sigma/\sigma_d = 1.3$$

L/U	280	290	300	310	320	330	340	350	360	370	380	390	400	410	N	M	σ	V
m	1			2	7	16	10	16	16	11	6	2	3	1	91	356	24	6.6
f		4	10	14	13	12	20	8	2	2	2	1			88	332	22	6.6

$$d_M/\sigma_d = 7.0 \qquad d_\sigma/\sigma_d = 0.8$$

S/U	540	560	580	600	620	640	660	680	700	720	740	760	780	N	M	σ	V
m	1	2	3	5	14	14	19	15	9	4	2	1		89	662	41	6.2
f			2	14	11	15	15	12	12	4			1	86	662	39	5.9

$$d_M/\sigma_d = 0 \qquad d_\sigma/\sigma_d = 0.5$$

The correlation between different measures of the same individual may be called organic correlation or self-correlation. The following table presents the male self-correlations in the upper half and the female in the lower;[5] a regular hierarchy exists, and the table has been arranged to show it:

	J	L	S	U	
J		.80	.70	.63	
L	.95		.76	.73	$M_{r_m} = .72$
S	.86	.89		.73	$M_{r_f} = .87$
U	.82	.83	.88		

The critical ratios (d/σ_d) for the sex differences between corresponding coefficients range from 1.7 to 2.7, and are therefore barely significant, as they stand. It is possible, moreover, that the superiority of the female correlations may be due to the greater variability involved. The usual formula for correction for range

$$\frac{\sigma}{\Sigma} = \frac{\sqrt{1-R}}{\sqrt{1-r}}$$

[5] All scattergrams encountered showed sensibly rectilinear regression, and all correlations are accordingly of the product-moment variety. The probable errors are .07 for all coefficients up to .32; .06 up to .49; .05 up to .62; .04 up to .72; .03 up to .81; .02 up to .89; and .01 up to .96.

assumes (since it was derived for use in connection with
reliability) that the standard deviations for test and re-
test in the same population are equal, and designates
them σ and Σ for the populations with small and large
variabilities, respectively.   The analogous situation
(equality of standard deviations for, say, J and L in the
female) does not exist here; but an approximation may
be made by using for σ the product of the two standard
deviations concerned for the male, and for Σ the corre-
sponding product for the female.   On this basis, and pre-
dicting the female self-correlation from the male, we find
the following estimated values:

	J	L	S	
L	.94			Average superiority to
S	.95	.91		obtained values, .05.
U	.92	.88	.93	

It therefore appears that the difference between male
and female self-correlation must be ascribed wholly to
the differences in variability.

The tetrad differences are of the order of one fifth
their probable errors, indicating a general factor; but
this is evidently merely the size of the animal.

Very slight self-correlation exists in the morphological
indices; the male coefficient for the indices J/S–L/U is
.07 and the female .14, probably chance values.

Cross-correlations are correlations between one mea-
sure in the male and another in the female; they may
thus be looked upon as additional measurements of ho-
mogamy, or, conversely, homogamy may be considered a
special case of cross-correlation.   Both are accordingly
presented in the same table:

		m				
		J	S	L	U	
	J	.47	.32	.29	.29	
f	S	.44	.31	.31	.25	M, = .29
	L	.40	.27	.20	.20	
	U	.29	.20	.16	.18	

Homogamy to the extent of about .3 is therefore defi-
nitely established in the animal studied.

Homogamy in the indices, however, is as definitely non-existent with the exception of one doubtful case; the simple homogamy coefficients are:

J/L	.19	J/U	.01	L/U	.04
J/S	.01	L/S	−.07	S/U	−.01

It may be concluded that so far as is indicated in the measures studied, homogamy is a function of size, not form.

Remating was frequent in the laboratory; while a strictly systematic survey was not made, check-ups at convenient periods showed 32 males and 30 females involved in 37 rematings. Five of the males remated twice; five of the females remated twice, and one three times; in one case a male was found clasped about another male, which in turn clasped a female. Homogamy coefficients for this remated sub-population were much reduced, although it should be observed that the numbers are small and that this is the severest possible test of the stability of the values in remating:

J	.23	S	.11
L	.15	U	.04

These are the values for males with their lowest numbered (*i.e.*, random, the numbering being in order of capture and measurement) remates; the coefficients for females with their remates differ inconsiderably. It is believed that the attenuation is due principally to the laboratory conditions of changed temperature and substratum, congestion and manipulation.

It has been established, then, that homogamy represented by a coefficient of about .3 exists in the toad, and that this homogamy is based upon size rather than form; that the animals are very highly self-correlated; that remating occurs under laboratory conditions, but homogamy is much reduced therein; and that there are marked sex differences in size, form and variability, the female being the larger and more variable.

The results of the principal investigation, that on homogamy, are in line with those from the only other vertebrate upon which data have been gathered—man— but (if physical traits alone be considered) slightly higher; and they are in contrast, as are the human data, to the results for invertebrates, which in turn are approximately consistent among themselves. The investigators of invertebrate homogamy are of the practically unanimous opinion that in the forms investigated by them the phenomenon is closely conditioned by the physical impossibility of copulation between animals widely divergent in size; this is particularly the case where the species is hermaphroditic (*Chromodoris*) or where for any other reason more than one point is involved (*Paramecium, Gammarus*), or where, only one point being involved, the apposed reproductive structures at that point are relatively inflexible (*Leptinotarsa*). The principal reason for the reduction of the coefficient in the two vertebrates examined is therefore the removal of these limitations, by the fact that the structures concerned are indefinitely flexible in man and that there is no intromission at all in the toad.

Why any degree of homogamy should remain is, however, still a problem. The existence of self-correlation explains adequately why structures remote from reproductive functioning (as forearm length in man) may show appreciable homogamy; nevertheless *some* structure or complex of structures must be sexually selected. In man some psychic (not necessarily conscious) basis appears to be the most promising hypothesis; for example, it seems likely that selection may be partly conditioned upon the heterosexual parent pattern, so that the mate resembles the parent because so selected, the selector resembles the parent by inheritance, and resemblance between selector and mate follows by the familiar Euclidean axiom. But this is hardly plausible for the toad.

Clinically, there appeared to be in our series an association between size, roughness, fatness, sluggishness,

intensitivity and possible color—a complex suggesting age differences. It therefore seems possible that mobility may be a factor; young females would be caught only by young males, old males would catch only old females, young males would catch all females indiscriminately, and old females would be caught by all males indiscriminately—a set-up which, *a priori,* seems adapted to bring about a low positive correlation. The next most promising hypothesis would seem to be a tactual or chemical one; and no doubt a very instructive result could be secured from the study of homogamy in, say, the stickleback, where mating, although definite, involves no physical contact whatever.

From the relative size of the homogamy coefficients in vertebrates and invertebrates it would appear to follow that the latter are forming sub-groups, at least with respect to size (Tower asserts, without offering evidence, that homogamy in color is absent in *Leptinotarsa*), much more rapidly than the former. This may be true; we have very little accurate knowledge of differentiation rates, but the enormous species frequency among, say, the Coleoptera, as compared with vertebrate groups of the same rank, would afford inferential evidence. Even though it be untrue in specific cases, however, the correct inference is probably that the effect of homogamy has been overridden by that of environmental selection; Fisher indicates in a recent work (9), for instance, that in many species the mean may be so delicately adjusted to the "niche" that considerable fluctuations either way are immediately wiped out; homogamy in such a case would have the effect of hastening the extermination of deviates, and no sub-groups would be formed unless a new niche developed. A third possibility, unexplored but highly significant, may be mentioned: Pearson has found that in man homogamy in stature is positively, but homogamy in eye color negatively, correlated with fertility. It is possible, then, that homogamy may work for

or against not only differentiation but survival, according to the trends coincident with it and the trait in which it occurs.

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A BIOLOGICAL STUDY OF A TEMPORARY POND IN WESTERN CANADA

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INTRODUCTION

THIS paper comprises part of the results of three years of field work (1925–27) on a temporary pond near Winnipeg, Manitoba, Canada. The term "temporary pond" is used with reference to small depressions in which water from the melting snows and spring rains collects and persists through the months of April, May and a part of June. Such ponds are characterized by having a relatively short period of submersion followed by progressively drier conditions and subject to low temperatures during the winter. Perhaps the most characteristic of the animals present is the phyllopod, *Eubranchipus gelidus,* the eggs of which require to be dried and subsequently frozen before they will hatch. As has been pointed out by Shelford (9) there is an extremely interesting seasonal succession in habitats such as this, involving very marked adaptation in the life histories of certain forms in adjustment to the peculiarities of the climatic cycle. No systematic study has ever been made of a pond of this kind in North America[1] and Shelford's paper (9) deals with an Illinois pond in which there was some water permanently, although there was a "temporary" area surrounding it. The present study, although far from complete, may therefore be of interest. During the spring and early summer of the years 1925, 1926 and 1927 the pond near Winnipeg was visited at least once each week and collections made. Casual observations had been made for some years previous to this. A certain amount of information relating to the physical and chemical conditions in the pond was collected but is

[1] Incidental reference has been made to ponds of this type in a number of papers, among which are those of Jewell (3) and Dexter (2).

reserved for a future paper after further observations have been made.

I should like to express my thanks for the assistance of certain specialists in determining a part of the collections. Those who have given especially valuable aid are the following: Dr. Ruth Marshall (Acarina); Dr. Chas. H. O'Donoghue (Bryozoa); Dr. J. W. Folsom (Collembola); Dr. Percy Moore (Hirudinea); Mr. J. B. Wallis (Coleoptera); Mr. Clyde L. Patch (Amphibia); Mr. Alexander Bajkov (Plankton). I also take great pleasure in acknowledging the help of Professor O'Donoghue in other ways, his encouragement having made this work possible.

DESCRIPTION OF THE POND

The pond upon which this work was done is situated between Abbottsford and Greenwood Avenues, a few hundred yards east of Saint Marys Road, in the municipality of Saint Vital, Manitoba. Some years ago, during a "boom" period in this district a large part of the countryside was subdivided and graded roads were built. Two of these cut off the extreme ends of a temporary pond, the central portion of which was examined during the present investigation. For the past five years (from 1925) or more, no further disturbance has taken place and no building operations have come within a quarter of a mile of the pond, so that it is still under natural conditions. Cattle occasionally wander through the pond but these may be considered the ecological equivalent of the buffalo which formerly inhabited this region. The original drainage of the pond is somewhat difficult to determine but probably never exceeded one mile in area. The maximum depth of the pond in the spring is about one meter. The water which forms the pond comes from two sources, the melting of the snow which accumulates during the winter and the spring rains. In this connection it may be mentioned that the city of Winnipeg which is not far distant has an average annual precipitation of twenty-two inches. The moisture conditions, however,

vary considerably from year to year. The spring of the
year 1925 and that of 1927 were rather more moist than
usual, while that of 1926 was rather dry.

From the standpoint of distribution, Saint Vital is
situated within that Forest-Grassland Transition belt
which Lewis, Dowding and Moss (5) have termed the
"Parkland." It may also be termed the Poplar Savan-
nah. This name is applied to the somewhat savannah-
like country which forms the belt of groves and glades
which extends from the Manitoba-Minnesota boundary
near Emerson, Manitoba, to the vicinity of the city of
Edmonton, Alberta, and southward from that point down
the foot-hills of the Rocky Mountains as far south as the
settlement of Pincher Creek, Alberta. In the vicinity of
the Saint Vital pond, as is the case throughout this re-
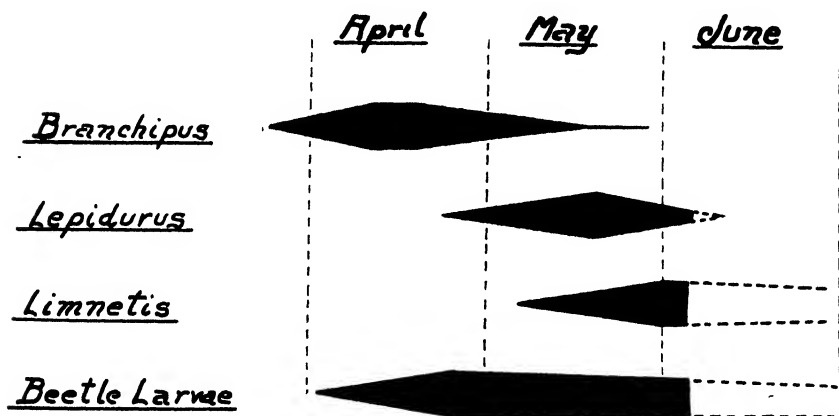
Generalized diagram of the seasonal distribution of some of the most
abundant animals in the pond. The relative abundance is indicated in a
general way by the thickness of the bars.

gion, the forest cover is composed largely of the poplar
or trembling aspen (*Populus tremuloides* Michx.) which
usually occurs in groves with intervening grassy glades.
In many places between the poorly drained belt of conif-
erous forests to the north and east of this point and the
dry southern and western plains, ponds or "sloughs"
are to be found which resemble the one which forms the
basis of this study. In fact this may be regarded as one
of the characteristic habitats of this region.

As is noted below there is a dense growth of herbs on the site of the pond after the disappearance of the water. During the summer these plants store up food materials, die with the coming of the frosts in the autumn and form the food of the animals during the following spring. In this way the herbaceous plants which grow on the site of the pond during the summer form the basis of the community, making possible the great abundance of animal forms the following spring. Thus in one sense the living organisms in this habitat never reach a state of equilibrium although they approach it. That is to say, the organisms are not in a state of equilibrium quantitatively, in that the relative abundance of the plants as compared with that of the phytophagous animals, and these in turn relative to the predaceous animals, could not be maintained season after season were it not for the temporary nature of the pond. The explanation of this lies in the fact that certain plant members of the community are able to exist (as a group) for five months or more in each year, whereas the phytophagous animals are compelled by natural conditions to remain dormant for all but two months. As a result of this there is sufficient food (which is the limiting influence as far as relative abundance is concerned), for a very large number of the phytophagous forms in the spring of each year. The same is also true of the predaceous animals.

In connection with the abundance of life in the pond for a short period in the spring of each year it may be worth while to note the remarks of Kofoid (4) regarding the plankton production of Flag Lake, near the Illinois River, in the vicinity of Havanna, Illinois. He states that:

The average plankton production of this lake, or, more properly speaking, marsh, . . . exceeds that in the river. . . . This greater fertility appears not only in the averages *but in general throughout most of the seasonal changes. Its run-off therefore serves generally to enrich the channel waters.

The greater production is due to the decay of the abundant vegetation which the lake contains, to the absence of tributary water of recent origin, to the relative freedom from the general current of outflow . . . and conse-

quently, to the greater time afforded for breeding an abundant plankton in this impounding area.

The dominance of the abundant vegetation is inimical to large plankton production. Other things being equal, plankton production is greater when the relative occupancy of the water by vegetation is decreased.

This is partly true of the Saint Vital pond, the great abundance of plankton organisms being the result of the decay of the vegetation, the lack of any outlet and the relatively high temperature. Pond (7) restricts Kofoid's principle to the non-rooted vegetation and states that the abundance of the plankton is in some direct ratio in proportion to the amount of the rooted vegetation. This being the case it is an additional explanation of the abundance of plankton organisms in the Saint Vital pond for a short period in the spring.

It is interesting in this connection to note that it is the practice in the carp ponds of Germany and Czechoslovakia to drain the pond when the annual production falls below a certain point. The following spring a crop of oats is sown on the site of the pond and after cutting the stubble is not plowed under. The spring following, the pond is again filled and carp introduced. The yield of the pond is greatly increased by this treatment. It will be readily seen that a very similar fertilizing process occurs under natural conditions in the Saint Vital pond and this is undoubtedly an important factor in the production of the exceedingly rich fauna, in so far as the number of individuals is concerned. It may be readily seen that this has important applications to fish culture and fish-food culture.

The conditions of existence in a habitat of this kind, especially in this northerly situation, are extremely rigorous. The winters are long and quite cold, temperatures of 30° to — 35° F. occurring every winter. Occasionally lower temperatures occur, the coldest on record being December 24th, 1870, when a temperature of — 58.5° F. was recorded in this district. The resting organisms are, however, protected by a thick blanket of snow but as the

frost penetrates the soil to a depth of seven feet below the sod, it will be seen that these forms must be exposed to moderately low temperatures. The snow usually begins to melt during the last days of March, and as a rule has disappeared from the woods early in the month of April. There is a great deal of variation in the time and nature of the thaw in any series of years. In the year 1926 nearly two weeks of bitterly cold weather succeeded the first thaw, with the result that ice of a thickness of three inches formed over the surface. This condition is shown on the accompanying diagram. In other years the
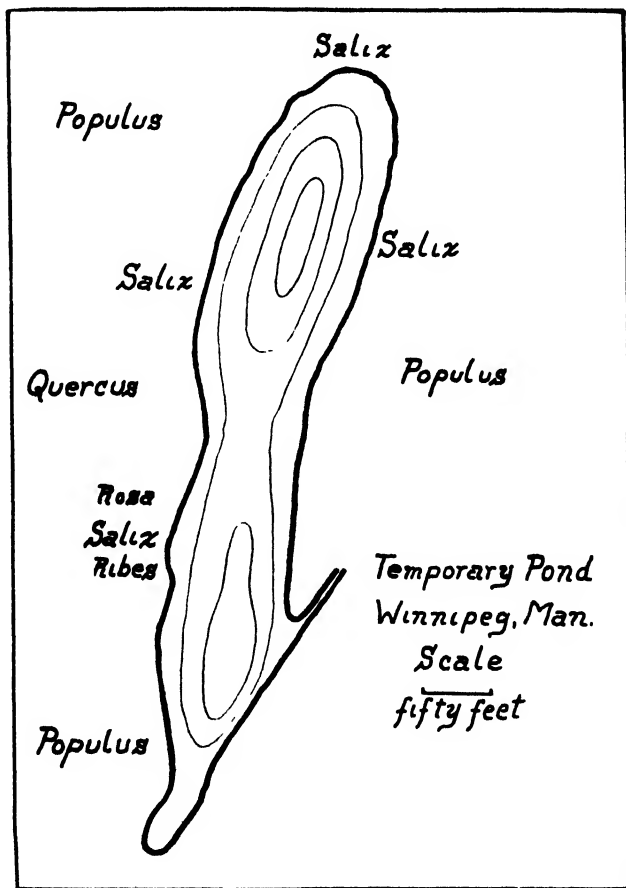
A TEMPORARY POND IN WESTERN CANADA

Map of the pond at Saint Vital, Manitoba. The deeper portions of the pond are indicated by the faint lines. Plane-tabled by R. D. Bird and Alan Mozley.

spring thaw sometimes comes suddenly and is succeeded by warm weather. With the advent of spring an extremely interesting succession of species commences as the various organisms hurry through their life histories before the drying of the pond, or, as is the case with *Eubranchipus,* before the appearance of their enemies. Those which appear very shortly after the melting of the snows include the collemboles, molluscs and adult beetles. The copepod, *Cyclops viridis,* also appear very early in the annual development of the pond. Among those which appear sometime later are *Lepidurus couesii, Limnetis gouldii, L. mucronatus, Diaptomus leptopus, D. oregonensis,* the Gerridae, *Notonecta undulata* and *Rana cantabrigensis cantabrigensis.* The date on which the pond becomes dry also varies from year to year, depending on climatic conditions, but this takes place as a rule between the first of June and the latter part of July. During the summer months succeeding this, the site of the pond becomes exceedingly dry and the soil often becomes parched and cracked. This is succeeded by the winter conditions described above.

It is interesting to note the various ways in which the organisms which live in the pond have adapted their life cycle to a common end, the maintenance of life during the dry period of the pond. This long dormant period of nine months or more is undoubtedly a great strain on the vitality of the organisms, and is probably one of the important limiting influences of the biota of the pond. Some forms such as the molluscs and frogs pass the winter in the adult form, while others, including the algae, fungi (*Saprolegnia*), Entomostraca and many insects pass the winter as eggs or spores. Still others, including the water boatmen (*Corixa*), water striders (*Gerris*) and the backswimmers (*Notonecta*) pass the winter in other habitats and migrate into the pond in the spring.

VEGETATION

The pond is surrounded by numerous large shrubby willows (*Salix longifolia* Muhl.). Immediately behind

these, where the level of the ground rises slightly above that of the pond bottom, a thick though rather stunted forest is found, consisting largely of the trembling aspen (*Populus tremuloides* Michx.) together with scattered oaks (*Quercus macrocarpa* Michx). Invading the pond in company with the willows are species of *Rosa* and *Ribes,* which have gained a foothold as a result of the slightly greater elevation of the central portions of the willow clumps. The pond area is devoid of trees and shrubs, except in a few places slightly elevated above the general level where a few very small willows are found. The portion of the pond which is submerged in the spring, while without shrubs, supports a large number of herbaceous plants, especially during the month of July. Among these there appears to be a definite succession, from hydrophytic forms, such as *Lemna trisulca* L., *Utricularia macrorhiza* Le Conte, *Riccia fluitans* (?), and an undetermined species of *Hypnum* in the spring, to more or less xerophytic species such as *Helenium autumnale,* and *Potentilla anserina* later in the year. Thus in the spring certain mesophytes develop rapidly under ⚹the favorable moisture conditions and complete their life cycle before or shortly after the drying of the pond. These form an interesting parallel with the animals. The plants which come within this category include the following species, *Mentha canadensis* (L) Briquet, *Ranunculus sceleratus* L., *Spirea salicifolia* L., *Milium effusum* L.?, *Glycera pratensis* L., *Beckmania erucacformis* (L.) Host, *Eleocharis* sp., *Thalictrum diocium* L., *Plantago* sp., and *Petasites* sp. When the water in the pond has completely evaporated, certain other plants, which thrive under comparatively dry conditions, continue development and flower during the later months. These include, *Aster paniculatus* Lam., *Helenium autumnale,* and *Potentilla anserina* L.

In connection with the vegetation it may be of interest to note the result of Shelford's work in the neighborhood of Chicago. He states (8, p. 174) that, ''In a forest cli-

mate when ponds become filled and drained they are oc-
cupied by forests. In the steppe climate they are occu-
pied by steppe or prairie. In the forest border area,
where our studies have been carried on, some ponds when
filled are occupied by prairies, others by forest. Dr.
Cowles is of the opinion that ponds with gently sloping
sides become covered with prairie, while those with steep
sides become covered with forest.''

Annotated List of the Species Present

It has been thought best to include the mention of the
various species present in the form of an annotated list
and to reserve the greater part of the information relat-
ing to the seasonal distribution of the various forms for
a later paper after additional observations have been
made.

Algae

Bacillariaceae

Stephaodiscus niagareae Ehrbg.
Mastogloia smithii Twaithes
Navicula obliquestriata Smith
Talellaria fenestrata Kutz

Melosira sp.
Amphora sp.
Nitzschia heufleriana G.
Ceratoneis arcus Kutz

Chlorophyceae

Closterium botrytis Meneg.
Spirogyra crassa Kutz
Tetraspora explanata Kutz
Stichococcus bacillaris Nageli
Botrydiopsis eriensis Snow
Bulbochaetae mirabilis Witt

Cosmarium cotrytis Nageli
Chlamydomonas sp.
Botryococcus braunii Kutz
Sphaerococcus sp.
Ulothrix zonata Kutz
Apiocystis braunians Kutz

Cyanophyceae

Anabaena flos-aquae Brebison
Spirulina major Kutz

Nostoc commune Vaucher

Protozoa

Trachelomonas volvocina Ehrbg.
Volvox perglobator Powers
Phacus pleuronectes Nitz

Eudorina elegans Ehrbg.
Bursaria sp.
Difflugia acuminata Ehrbg.

Bryozoa

Fredricella sultana Blumenbach

This species is not particularly common in the Saint
Vital pond, but specimens have been collected on a num-

ber of occasions. *Fredricella sultana* has also been found in a temporary pond near the crossing of the main line of the Canadian National Railways with the "Southwestern Branch" of the Canadian Pacific Railway, a short distance west of the city of Winnipeg.

Hirudinea

Glossiphonia fusca Castle

It is interesting to find this soft-bodied animal as an inhabitant of a temporary pond. It is quite common, several individuals being collected on each visit almost throughout the period of this study. Dr. Moore states that this species commonly feeds upon snails and less frequently upon worms and insect larvae.

One, and possibly two, species of turbellarid worms have been collected in the pond but have not been determined.

Rotaroria

Diplax videns Levander	*Colurus grallator* Gosse
Salpina spinigera Ehrbg.	*Diplois daviesiae* Gosse
Pterodina patina Ehrbg.	*Brachionus angularis* Gosse

Crustacea—Phyllopoda

Eubranchipus gelidus (Hay)	*Limnetis mucronatus* Packard
Lepidurus couesii Packard	*Limnetis gouldii* Baird

The phyllopods listed above are among the most abundant and characteristic animals found in the pond. The *Eubranchipus* develop the most rapidly after the spring thaw and females with eggs are usually very common about one month after water first collects in the pond in the spring. *Lepidurus* usually attains its maximum abundance about two weeks later, while the adult *Limnetii* appear in numbers about two weeks subsequent to this, that is to say about the first of June. *Eubranchipus* is extensively preyed upon by the larvae of the beetles noted below.

Copepoda

Cyclops viridis Jurine	*Canthocamptus minutus* Claus
Cyclops ater Herrick	*Diaptomus leptopus* Forbes
Diaptomus oregonensis Lilljeborg	

Cladocera

Simocephalus vetulus Mull.   *Ceriodaphnia reticulata* Jurine

Ostracoda

Cypris virens Jurine   *Cypris dentata* Sharpe
Cypris fuscata Jurine   *Candona* sp.

Insecta

Neuroptera, Hemiptera and Coleoptera are the most abundant insects in this pond. In general they appear for the first time in the spring rather later than the molluscs and crustaceans.

Collembola

Isotoma viridis Bourlet   *Tomocerus   flavescens   americanus*
Isotoma palustris Muller   Schott
                         Entomobrita sp.

These species are found on the surface of the pond in great abundance soon after the spring thaw. They are extremely numerous for a few weeks but later disappear almost completely.

Hemiptera

Gerris rufoscutelatus Latr.   *Notonecta undulata* Say
Gerris buenoi Kirk   *Corixa* sp.

All these forms migrate onto the pond some time after it is free of ice in the spring. The *Notonecta* is sometimes found quite abundantly and at others is very scarce. The Corixidae, of which there are probably several species, occasionally migrate into the pond in enormous numbers.

Coleoptera

Hydrophorus fuscipennis Kies   *Illybiosoma bifarius* Kirby
Agabus punctulatus Aube   *Colymbetes sempltilis* Hart
Agabus triton Fall   *Gyrinus maculiventris* Lec.
Agabus sharpii Fall   *Beroses straitus* Say
                Hydrocharis obtusatus Say

These beetles are quite common in the pond. Some individuals are to be found in the spring immediately after the melting of the snow, while there is still ice in the pond and the water is very cold. They apparently lay their eggs early in April and the young larvae begin to

appear soon after. These small larvae have been observed feeding upon some of the Entomostraca. When they are somewhat larger they commonly prey upon the *Eubranchipus* and when the latter have all disappeared the beetle larvae feed upon the tadpoles of *Rana cantabrigensis.*

Caddice larvae (Trichoptera) are common, but the species have not been determined. This is also true of the mosquitoes. The larvae of damsel flies (Odonata) have been collected on two occasions but their occurrence was undoubtedly accidental and they do not reach maturity in the pond.

Hydracrina

Panius cataphractus (Koenike)	*Eylais desceta* Koenike
Hydrachna legei Koenike	*Eylais* sp.

These water mites are common in the pond. The microscopic larvae have been found early in April, but the adult forms do not appear until five or six weeks later.

Mollusca[2]

Special attention has been paid to the molluscan fauna of the pond. The following list is believed to be complete:

Lymnaea palustris Muller	*Planorbis exacuous* Say
Lymnaea caperata Say	*Segmentina crassilabris* Walker
Planorbis umbilicatellus Cockerell	*Segmentina christyi* Dall
Aplexa hypnorum Linne	

Lymnaea palustris is by far the most abundant mollusc of those found in the pond. When the pond dries in the month of June the snails secrete a diaphragm across the aperture and remain dormant on the bottom of the pond, in cracks and small depressions, until the following April, and thus have an inactive period of between nine and ten months. It is somewhat remarkable that any species are able to survive year after year, when they are able to be active and to feed during only two months out of every twelve.

[2] See also Mozley (6).

Planorbis umbilicatellus is not particularly common in the main portion of the pond. Ten or twelve individuals of this species were usually collected on each visit, while several thousand individuals of *Lymnaea palustris* could have been collected with very little effort. In a similar but rather smaller and more shallow pond situated a few yards south of the one studied in detail, with which it was formerly connected, *P. umbilicatellus* was extremely abundant, so much so that fifty or more specimens were often scooped up in a sweep of the collecting net. The reason for this great difference in abundance is not apparent. Observations on the snails and bivalves of a number of similar ponds in Manitoba and Saskatchewan are at present in progress and a more detailed account of this smaller pond will be given after additional observations have been made.

There is a great deal of variation in the form of the shell in *Lymnaea palustris* Muller, as found in the pond. It is interesting to find this wide range of variation, from short obese individuals to those of a slender narrow type, in one habitat. Baker (1) has pointed out that in any considerable series of shells of this species from one locality, there is a very wide range of variation. But in the case of the Saint Vital pond there is an equally wide variation not only in shells from one locality but from one habitat. That is to say, in this very restricted habitat in which the conditions are very nearly uniform throughout, there is quite as wide a variation in the form of the shell as is shown in most series from many different localities. Obviously, therefore, the variable factors which give rise to the differences in the form of the shell in this species under these conditions are largely internal. This fact is undoubtedly well known to many malacologists although it has apparently not been definitely stated up to this time. Several previously published statements may refer to this, but the wording is so muddled as to be quite impossible to understand.

Amphibia

Rana cantabrigensis cantabrigensis (Baird)

This species, commonly known as the "Northern Wood Frog," is the only frog found in the vicinity of the pond. It is quite abundant and is a characteristic member of the pond fauna. The leopard frog, *Rana pipiens,* which is common practically throughout the Province of Manitoba, does not occur in this habitat. The wood frog appears in the spring soon after the snow has disappeared. It was first noted in 1925 on April 3rd; in 1926 on April 18th, and in 1927 on April 15th. The tiger salamander (*Amblystoma tigrinum*) which was found by Shelford in a pond near Chicago is not a temporary pond form as it requires some "permanent" water to reach maturity.

Aves

The presence of the pond does not seem to have any very marked direct effect upon the avian fauna of the vicinity. A few ducks are often found feeding there early in the spring but these are merely migrating individuals. A bittern has been observed feeding in the pond on two occasions. The typical shore birds are absent from the pond, probably as a result of the lack of an open shore free of plants, and the temporary nature of the pond.

Summary

A temporary pond in southern Manitoba, Canada, situated at fifty degrees north latitude and ninety-seven degrees west longitude, was studied during the spring of the years 1925, 1926 and 1927. This pond was formed by the melting snows and spring rains in the month of April, and usually persisted until sometime in the month of June. The period of activity of the aquatic animals which are permanent residents of the pool, not merely migrants, is thus somewhat more than two months in each year. During the summer, after the pond has be-

come dry, the dormant organisms are subjected to severe desiccation and during the winter to low temperatures.

The flora and fauna of this pond is quite extensive, and many of the species present occur in very large numbers. The phyllopods, *Eubranchipus*, *Lepidurus* and *Limnetis*, beetles and snails are the most numerous macroscopic forms. There is a well-marked seasonal succession in both plants and animal species. The alternation of moist and dry conditions plays an important part in the great productivity of the pond and has some possible applications to fish culture and fish-food culture.

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APPARATUS AND METHODS FOR DROSOPHILA CULTURE

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A FOUR-SHELF INCUBATOR FOR DROSOPHILAS

DURING the early work on the genetics of *Drosophila melanogaster* the cultures were reared on laboratory tables or on open wall shelving. This method gave irregular results. The cold nights of the winter season made a considerable proportion of the cultures fail to start properly, and also lengthened the generation interval.

In 1913 I built above my laboratory table a large wooden incubator which held all my experimental cultures. This incubator was heated by carbon electric lamps placed in the lowest shelf space. The thermostat was of the "ether-wafer" type. The expansion of the ether forced apart contacts in the heating circuit. A six-inch electric fan continuously circulated air down a gap behind the shelving with return through a similar gap between the shelves and the doors.

Shortly after this, two other large incubators were equipped for other workers. In these the thermostat contacts controlled a relay which turned the lamps on and off. Plough's work on the effect of temperature extremes on crossing-over showed that it was necessary to maintain the temperature constant for genetic reasons as well as to improve culture conditions.

In 1922 a large incubator with four shelf spaces was designed and built. It has operated since with marked success. Its construction is of inexpensive materials and is simple enough to be made by the investigator or any ordinary carpenter. It is capable of maintaining accurately temperatures below room temperature as well as above (but not simultaneously). Its flexibility and range are great enough to provide any temperature needed in the Drosophila work. Most of this falls between 13° C. and 31° C., but occasionally still lower or

higher temperatures are required. The humidity is controllable, and may be held constant for many days without attention. The construction is such that great changes in humidity can be brought about within the incubator without trouble from warping or opening of seams. The entire interior of the shelf compartment is at substantially the one temperature for which it is set, without local hot spots, and without the vertical temperature gradient that is so marked a feature of most incubators. In the account which follows, and in the accompanying plan and elevation diagrams, the original design has been improved in a few particulars, which will be specified.

Uniformity of temperature within the incubator chamber is attained as follows (see diagram):

(1) No source of heat is within the incubator chamber. The six heating lamps are in a separate compartment below, and the incubator space is shielded against radiation and conduction from them by interposing four wooden partitions and three streams of air. The heat of the lamps is applied to the outside of the incubator chamber, and only in the form of a rapidly moving air bath of regulated temperature. The tenth-H. P. motor, which drives this air stream by an eight-inch fan, gives off considerable heat, and hence is likewise placed in a separate compartment. The motor compartment is well insulated from the incubator chamber and from the air stream, and is itself ventilated by outside air through groups of holes in its door.

(2) The stratification, with vertical temperature gradient, which exists in the air of a laboratory, and which is contributory to the temperature gradient within the average incubator, is done away with by the fact that a regulated air bath is interposed between the room and the incubator chamber. The regulated air is forced to cover all the wall surface, even the front, by dividing the three-inch (originally four-inch) space between the incubator chamber and the outer casing into connecting

1 foot
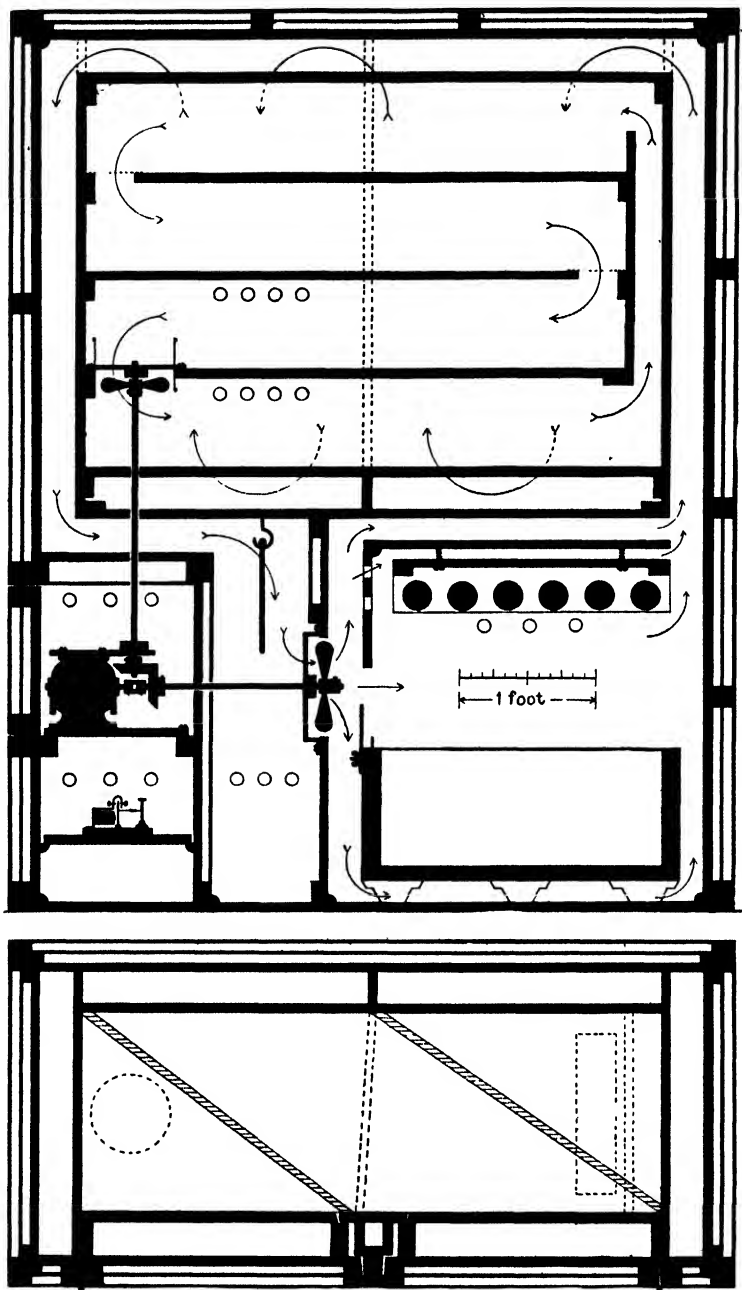
passages which form a closed air-duct system. The air
passes from the fan through the various channels in the
large tempering chamber, then up the right end, diago-
nally across the top, down the right half of the back,

forward below the chamber, up through the right door and the hollow center post, diagonally across the top to the left half of the back, down this, forward beneath the left half, up through the left door, diagonally across the top, down the left end, along the bottom to the thermostat compartment, and is finally forced through a nine-inch hole into the tempering chamber again. Most of this movement is in an up-and-down direction, which in itself tends to equalize the stratification effect. In the incubator as built in 1922 the air did not pass through the doors, but instead made two return trips, down and up and back. In the new design, with a spiral course for air in the walls, there are no corners to form dead air spaces, and the entire outer wall of the incubator chamber is covered by a uniform flow of air.

(3) The interior of the incubator chamber is converted into a continuous air duct by the dividing shelves, which contain screened connecting openings at alternate ends. The circuit is completed by a vertical return passage, at the right end of the chamber, connecting the bottom with the top shelf space. The air inside the incubator chamber is all kept in motion and is driven in succession through all parts of the chamber by a continuously operated six-inch fan which receives its power from the same motor that drives the outer air stream. The fan blades run in a short vertical tube let through the left end of the shelf that forms the roof of the lowest space. The air streams inside the incubator chamber are mainly horizontal, and by cross-hatching the vertical streams in the walls equalize still further the temperature gradients.

Both these air streams are driven at high speed, since air has a low heat-carrying capacity. To operate silently at full motor speed the shafting must be straight and of generous diameter ($\frac{1}{2}$-inch), and the bevel gears true and smooth. To facilitate putting in the 28-inch vertical shaft, the motor shelf and the relay shelf below it are removable. They are only eight inches wide, and the motor shelf is adjustable by washered screws in slots to

assist in aligning the shafting. A door, about a foot square, in the left end, enables the motor to be installed, adjusted and oiled easily. The bearings, originally thick brass, may be hard wood. Oiling of the bearings and the gears may be mainly graphite with just enough grease to keep it from flying. Graphited felt pads pressed lightly against the shafting near the bearings by adjustable springs further silence the system (not shown). Aside from the slightly greater expense, it would be better, and more silent, to dispense with the gearing and run the vertical shaft by a second motor placed above the other in the same compartment.

After making a circuit through the wall passages the air returns to the thermostat chamber, where, if it is cooler than the temperature set, it causes the lamps to go on until the set temperature is regained. For a large differential between the room temperature and a high operating temperature, the lamp load would be great enough to make it advisable to use a relay between the thermostat circuit and the lamp circuit. In the diagram the operation of the lamps is shown as controlled by a relay and a toluol-mercury thermostat in the form of a grid suspended from the floor of the incubator chamber. But this arrangement is optional, since there are so many desirable heat-control systems that this feature is not treated critically in the present paper. The use of a Thyratron tube as a relay is apparently the best solution of several major difficulties (F. O. and O. H. A. Schmitt, *Science, 73*: 289).

Since there is considerable consumption of oxygen and liberation of CO_2 when the incubator is full of cultures, an arrangement was made for renewing the air inside the incubator chamber. On the low-pressure side of the 6″ fan a row of holes took air from the duct behind. On the high-pressure side another row of holes let an equivalent amount escape. By stoppering one or more holes with corks, the speed of interchange could be regulated. Originally leakage from the outside was depended upon

to renew the air in the tempered stream, but, following the suggestion of H. H. Darby, sets of holes admit fresh air to the bottom of the thermostat compartment and emit it from the tempering chamber at the level of the lights.

Moisture is supplied by evaporation from the surface of a large-area tray ($25'' \times 22\frac{1}{4}''$) set above the bottom of the tempering compartment. As the water evaporates, the supply is renewed continuously from four (or two) five-gallon bottles (not shown), each inverted on a large tripod ring. The heights of the tripods are such that the mouths of the different bottles all come one and a half inches above the floor of the tray—a height great enough for the corks to be extracted after the bottles are inverted into place. The size of the standard five-gallon bottle is $19\frac{1}{2}$ inches in height by 10 inches in diameter. The clearance between the lights and the tray bottom is 23 inches, and more clearance can be obtained temporarily by unscrewing the lamps or the entire lamp-board (three screws). The percentage humidity can be cut down, from that corresponding to full exposure, by covering the tray top with boards (not shown) laid edge to edge, beginning at the fan side. The width of the boarding must be determined by trial, with humidity measurement at the particular temperature being used. The large volume of the reserve supply and the constant exposure surface enable the humidity to be kept constant for a very long period without attention. In renewing the supply in the bottles it is best to use water preheated to the incubator temperature.

For use at or below room temperature, ice, instead of the water bottles, is put into the large tray. This tray is deep enough ($10\frac{1}{2}$ inches) to take the standard thickness (10 inches) of artificial ice, and large enough (inside dimensions $= 22\frac{1}{4}$ inches from front to back, 25 long) to hold 150 pounds (standard 310-pound cake measures $10 \times 22 \times 40$ inches). The tray is strongly made, the bottom, ends and front side being two layers of the $\frac{3}{4}''$

material crosshatched, while the back is one thickness only. It is lined with heavy-gauge sheet copper. It may be provided with an overflow pipe (not shown) placed about two inches above the bottom and carried through the end wall.

For maximum cooling effect an adjustable partition ($\frac{1}{4}''$ beaver-board) at the left end is raised enough to divert a third or more of the air down the left end, under the tray (supported on strong legs $1\frac{3}{4}''$ high) and up the right end. For less cooling this partition is lowered, or turned horizontal to block off entirely the passage beneath the ice-box. For still less cooling, the setting being at or only slightly below room temperature, boarding (not shown) can be laid edge to edge from the fan end toward, or to, the opposite end. Without these diversions and blanketing the ice would be used rapidly in proportion to the height of the temperature setting, instead of inversely.

The boarding and timbering throughout is of $\frac{3}{4}''$ pine or fir, as soft and light weight as possible. This gives better insulating properties and an incubator easier to move about. The boarding is narrow—not over five inches wide, and is tongued and grooved. The timbering, or crosspieces, is of $\frac{3}{4} \times 1\frac{3}{4}$-inch material.

To prevent warping and opening of cracks from changes in humidity a special system of nailing is used. In making up a panel, the first or outer board at the ends of the crosspieces is nailed to the crosspieces by pairs of nails $\frac{3}{4}$ inch in from the outer edge of the board. The inner edge is left free. The next board is put in place but is not driven snug to the other; a crack of $\frac{1}{8}$ inch or less, depending on how dry the board is, is left between. This crack is of course sealed by the tongue and groove edge. This second board is nailed by pairs of nails at its midline. The successive boards are loose-laid and middle-nailed in the same fashion until the last or outer board is reached and this again is nailed near its outer edge only. Upon absorbing moisture, or on drying,

boards change in length very little, as compared with the change in breadth.   Hence, on the above system of nailing, each board is free to swell or shrink in place without opening cracks.   The over-all width of the panel depends only upon the amount of swelling in the length of the crosspiece, which is of the same proportionate amount as the change in length of the panel.   In nailing to this panel, *e.g.,* other crosspieces or members, this same rule is to be followed—nail into outer edge of outer boards; into the middle of middle boards.   The slight amount of leakage through the tongue and groove boarding is an advantage rather than otherwise, since it gives a diffused renewal of air.

The outer wall is covered completely by $\frac{1}{4}''$ cardboard in two successive layers.   Air spaces of $\frac{3}{4}''$ were made between the boarding and the cardboard by slats of $\frac{3}{4} \times 1\frac{3}{4}''$ material at the edges and horizontally between. Some of the modern insulating boarding (Celotex, Masonite, etc.) would be better.   This is mostly about a half inch thick, but can be substituted throughout without change in the dimensions for the wooden construction.

The upper doors (shown in plan) are of the same hollow construction as the back and ends of the incubator. The tops of the doors are level with the upper edge of the top board of the incubator chamber.   The bottom edges are level with the bottom edge of the bottom board of the incubator chamber.   The open bottom end and the open top of each door fit openings in the sill and lintel, leading into the cross passages below and above the incubator compartment.   The doors close against facings of $\frac{1}{8} \times \frac{3}{4}''$ soft felt weather stripping.   Wherever necessary quarter-rounds (not shown) are used to carry the facing.   Again, slight leakages are not a disadvantage.

The lower doors (not shown) are simple—$\frac{3}{4}$-inch boarding, with the crosspieces outside and covered over by the cardboard, leaving $\frac{3}{4}$-inch air spaces.   The top line of the lower doors is at the bottom of the top board of the motor

compartment.   The bottom line is at the top of a 4½-inch baseboard (not shown), having the same construction as the lower doors.   The hinge lines are at the inner edge of the side boarding of the casing.   The free edges meet on a stepped-back middle post at the front edge of the fan partition.

In assembling, the wooden part of the outer case is first built with the motor compartment and partitions, but no front, and is laid on its back.   The incubator compartment is built similarly, with the cross passages beneath and the projecting partitions behind and above, and is then lowered into place in the casing.   The front, with the doors, is then built in place, joining these two. Lastly, the outer cardboard coverings are applied over supporting slats.

A TWO-SHELF INCUBATOR FOR DROSOPHILAS

For many purposes neither so capacious an incubator as that described above for Drosophilas nor one able to run below room temperature is required.   The most efficient form for a smaller incubator seems to be two relatively long shelf spaces rather than more but shorter shelves.   The special advantage of this arrangement is that it minimizes the vertical temperature differences due to the gradient in the room.   Incubators with two long shelf spaces have also been built by B. Cohen, C. R. Plunkett and C. I. Bliss, and many of the later designs have had the benefit of consultation with other members of this group.

The interior is divided horizontally by a shelf, with a supporting partition at each end.   One of these partitions (see diagram) is complete below, except for a circular hole for the eight-inch fan blades.   The other partition reaches only to within five inches of the floor, except at its sides, where two-inch pieces act as legs for the shelf.   This end partition is set four inches from the end wall and forms an air passage to the upper shelf space.   It reaches halfway to the roof, thus permitting
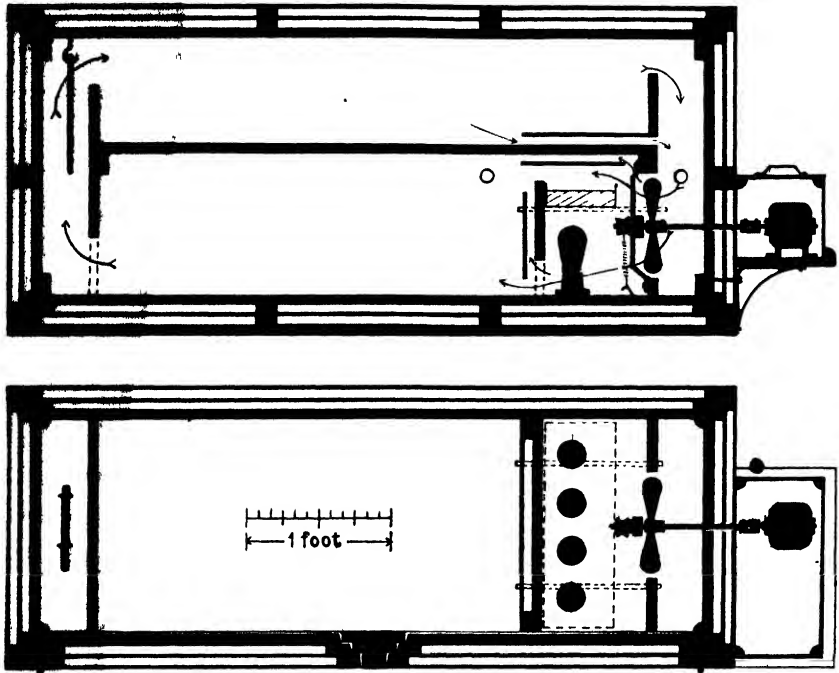
the air to flow while keeping bottles in position. In this end passage is hung the thermostat.

The fan is run continuously by a motor placed outside on a shelf at the end. This shelf is made large enough to hold the relay or other devices used in the heat control, and may be provided with a detachable cover which reduces the noise from the motor. The cover must be ventilated so that the motor does not overheat.

The heating lamps are in a special compartment next the fan in the lower shelf space. They are in series two-by-two, to operate at half voltage. Two such gangs provide the total wattage required. The two lamps in series with each other should be of the same wattage. The total wattage should be adjusted as low as will just overcome, by continuous burning, the difference between the chosen inside temperature and the minimum outside tempera-ture expected.

Since the heating elements are inside the incubator chamber, very special precautions must be taken to avoid higher temperatures next this compartment and in the

shelf space immediately above it. This is accomplished
by a succession of partitions with the interspaces swept
by air streams. Directly above the lamps is interposed
a metal tray for water. This rests on $\frac{3}{8}''$ removable dowel
pins that cross the heating chamber. The tray sets
snugly against the partition away from the fan and ex-
tends toward the fan, leaving a passage for about one-
third of the air to cross the water surface and pass over
the partition, which rises only to the level of the top of
the tray. Above the water tray is a horizontal partition
of plywood that is hung by screws to the middle shelf
or nailed to narrow strips parallel to the air stream.
Finally, the bottles on the shelf directly above do not rest
upon the long main shelf itself but upon another plywood
horizontal short partition raised upon narrow strips so
that an air stream passes beneath it and into the return
passage at the end. The top part of the end partition is
mounted on this plywood to provide exit for this air.
The partition separating the tempering chamber from
the rest of the lower shelf is similarly reinforced by an
accessory plywood partition so placed as to divert air
through the space between it and the main partition.

The humidity can be controlled by covering over the
water tray to an extent determined by trial.

The construction of this incubator is especially easy.
The inner case and horizontal shelf and main partitions
are made of $\frac{3}{4}''$ matched lumber about four or five inches
wide. The framing crosspieces are of $1'' \times 2''$ scantling,
which becomes $\frac{3}{4}'' \times 1\frac{3}{4}''$ when dressed on all four sides.
All this material should be light-weight soft pine or fir,
which is better as an insulator and gives an incubator
which is easier to move about. A special trick in the
nailing prevents changes in humidity from affecting the
structure. The boards are matched a little loosely and
are nailed at the outer edge only in the case of the out-
side boards of a panel and at the mid-line only in the case
of the central boards of a panel. The width of the panel
then depends only on the change in length of the cross-

members, which is the same as the general change in the
length of the boarding.

The insulation is easily added in two successive layers
over ¾ × 1¾ scantlings at the edges and horizontally be-
tween edges. Quarter-inch builders cardboard or the
thicker and better Celotex or Masonite can be used.

Slight leakages at the door cracks provide a diffused
exchange of air, which can be increased by holes bored,
at the level of the horizontal scantling, on the positive
pressure and on the negative pressure side of the tem-
pering compartment.

A TOLUENE-MERCURY THERMOREGULATOR

Parallel to the series of incubators, developed for use
with Drosophilas, has been a series of thermostats. The
thermoregulator described and diagrammed herewith is
the current model. As in the case of the incubators,
these thermostats have incorporated the criticisms and
improvements suggested by a large group of users, in-
cluding especially C. I. Bliss and C. R. Plunkett.

In general form this thermostat (see diagram) is a
grid of six parallel tubes filled with toluene (toluol) and
connected to a strong crosstube which terminates in a
mercury trap. The tubes are about 9½ inches long, ⅜
inch in diameter and fairly thin-walled. The crosstube
is heavy-walled, a half inch in diameter and about five
inches long. The mercury trap is of ⅜ inch heavy-walled
tubing. The near arm descends parallel to the six thin-
ner tubes and bends back to terminate in a vertical capil-
lary tube. The capillary tube has very thick walls and
a large bore (2 mm). This large bore gives a solid mer-
cury column that does not clog or cling to the walls, as
it would in a smaller bore. The sensitivity, reduced by
the large capillary, is made large by the great length and
large surface of the toluene grid. The movement of the
mercury column is well over an inch for a temperature
change of one degree centigrade.

1 inch
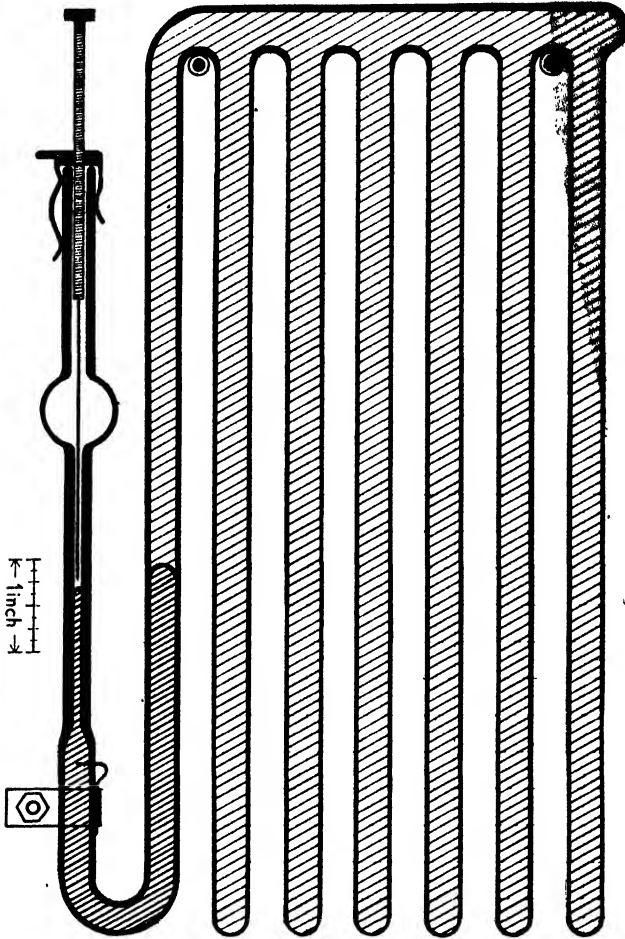
As the mercury column rises with increase in temperature it makes contact with the tip of a three-inch, half-millimeter diameter, nickel-silver wire. This wire is carried by a three-inch 6–32 brass machine screw with fillister head. The screw runs in a nut which is held in place at the top of the trap arm by a strong spring clip to which it is soldered. The arms of the spring clip are grooved on their inner faces to make the alignment positive, and hold firmly by virtue of a three-contact system, two contacts on one arm and the third on the other.

The electric attachment is made to a projecting lug on this spring clip by a toothed end-clip that terminates the lead-in wire. The other electric attachment is made by

a similar end-clip to the projecting ends of a copper band that encircles the tube below the capillary. This band is bolted firmly in position, pressing against a platinum or tungsten wire which pierces the wall and enters the mercury in the trap. The platinum contact wire was found to be too weak to endure direct attachment, hence the attachment band to take the end-clip.

The regulating screw is carried in a larger thick-walled tube above the capillary proper, only the long terminal wire entering the capillary. This arrangement was adopted to avoid change in the setting if the mercury should rise above the contact point. The space between the screw threads and the capillary wall would retain considerable mercury when the temperature fell again.

Between the capillary and the screw-carrying tube is blown a ¾-inch bulb, which acts as a reservoir for excess mercury. With this bulb present, the thermostat may be operating at 10° C., and when brought out into the laboratory at 25° C. the mercury will rise into the bulb and upon replacement at 10° will fall again without change in the setting. Or, a thermostat set for 25° C. could rise to 40° C., through failure of the heat to turn off, and mercury would not be forced to overflow the top.

The grid is freely suspended on two large cup-hooks covered by rubber tubing. One of these hooks must be in the space nearest to the mercury trap because of the overbalancing effect of the mercury.

The grid is best filled by toluene through a special capillary funnel or pipette extending through the capillary stem into the trap chamber below. When this chamber is filled the fluid can be passed on to the grid by tilting. The upper corner, where crosspiece meets trap tube, is well rounded, so that air bubbles can be easily removed by tilting. After the grid and trap are completely filled with toluene, the mercury can be added similarly, and will displace the toluene if the grid is tilted correctly between additions. The mercury should rise in the trap arm to about the middle of the capillary height.

A weak point of this thermostat system is oxidation of the mercury surface through sparking, when a 110-volt current is made and broken. This can be reduced below the trouble level by increasing the capacity of the line by connecting the light circuit and the fan circuit in parallel with the thermostat gap, so that the surge has an open path. Another method is to reduce the voltage in the thermostat circuit and employ a relay that is wound to operate on the given small current. Dry cells, storage batteries, the plate current from a vacuum tube (the system given by D. J. and J. J. Beaver, Ind. Eng. Chem. 15, 359) or other means may be employed to give a low voltage circuit. The use of a Thyratron tube apparently offers the best solution of this difficulty, as well as of several others (F. O. and O. H. A. Schmitt, *Science,* 73, 289).

The large travel of the tip of the mercury column for a change of one degree centigrade allows the operating range to be as small as about $\pm 0.01°$ C. However, the heating system usually has a lag great enough to make the temperature overshoot in both directions. The actual operating range is nearer ± 0.05 and may be greater. Thus, obtaining a narrow operating range does not require a thermostat more sensitive than this, but a more delicate adjustment of the heating system.

For minimum range the heaters must have minimum lag. Coarse resistance wires have considerable lag between the make of the current and the development of heat to full capacity, and much greater lag in the cooling after the break. Any kind of core increases the lag over open coils of bare wire. Small wires operated at high capacity are better than large wires operated at low temperatures. Electric lamps with fine filaments in a vacuum are most excellent, as well as very safe in operation. But to avoid disturbance through burning out of lamps it is my practise to put them in series, two-by-two. This method operates them at half voltage, with a manyfold increase in life and dependability. Two or more of

these gangs of lamps are run in parallel to give the total wattage required.

A second factor in small-range operation is smallness in the amount of heat turned on and off by the regulator. The smaller the difference between the incubator temperature and the exterior temperature the smaller is the variable heat unit needed. Also the smaller the fluctuation in outside temperature, especially drops below incubator temperature, the smaller the heaters can be made. The optimum arrangement for very fine work is to operate the incubator in a room which is itself regulated to a temperature about two degrees, or even less, below that of the incubator.

A large but rather uniform difference between outside and inside temperature may be met by increased insulation or by use of accessory lamps which burn continuously and are adjusted in wattage to bring the inside temperature to within three to five degrees of the temperature desired.

CULTURE BOTTLES FOR DROSOPHILAS

In the early work on the heredity of Drosophila the cultures were raised in quart fruit jars, in quart milk bottles, and in a very miscellaneous collection of museum jars and other laboratory glassware. The great difficulty was in getting the flies out of these cultures for etherization and examination. By 1913 pint and half-pint milk bottles had become standard, largely on account of their uniformity of mouths, their heavy, strong glass and the ease with which they could be procured.

In 1916 I made experiments to determine the optimum area of culture surface, and concluded that the pint milk bottle was best for the flies, but the half-pint, which was not far inferior in that respect, was greatly superior in ease of handling, etc. Accordingly in 1917 I drew designs and made a wooden model of a special bottle, having the floor area of the pint bottle and the height (135 mm) and other good features of the half-pint bottle. A

special feature of this model was that the bottom was square, with generously rounded corners, and the sides were pyramidal with rounded angles. The angle between the bottom and the sides was to prevent the food cake (banana-agar at that time) from slipping out of place on the bottom, as it easily did with the cylindrical-sided milk bottle. Unfortunately, this model was never made in glass.

In 1925, Curt Stern visited our laboratory and was interested in culture-bottle design. Upon his return to
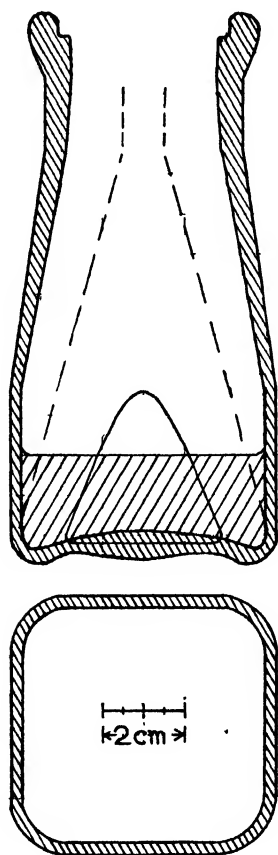
Germany he designed and had made a conical type of bottle, which had extra safeguards against displacement of the food cake by projections from the inner wall. The large size of the circular bottom seems a slight disadvantage of this excellent design.

In 1930 I again made designs for a culture bottle for
Drosophilas (see diagram), and the mould and bottles
were made by the Illinois Pacific Glass Company at their
plant in San Francisco. The mould is interchangeable
with one of the standard moulds on an automatic mul-
tiple-mould machine, which makes many half-pint bottles
simultaneously. In this way they are able to offer the
special bottles at the standard price of half-pint milk
bottles, if ordered in lots of five or more gross.

The features of the new bottles, which have been sub-
jected to a year's trial, are the following:

The glass is heavy and strong like that of half-pint
milk bottles, which are subject to hard usage.

The mouth is the standard milk bottle mouth, with the
groove for paper caps. This type of mouth was adopted
for two main reasons: the groove is convenient in receiv-
ing and holding the entrance funnel of an etherizer with-
out danger of a slip. Also it seems likely that the paper
caps regularly used for milk may ultimately entirely dis-
place the cotton plugs with cheesecloth covers that have
been standard since 1921. The use of the paper caps was
begun by Demerec in 1928. But my own tests of them
indicate that better results are obtained if they are per-
forated with ten or fifteen small needle holes than if they
are used imperforate as originally suggested. A gang
punch could be made to perforate several at one lever
stroke. The holes should be numerous and not over 0.6
mm in diameter, otherwise undersized Drosophilas can
enter or emerge.

The neck is cylindrical for about 2 cm below the neck.
This gives a long seat for cotton plugs, which do not pop
through as they did with milk bottles whose flare begins
just below the mouth.

The bottom is square with rounded corners. The short
diameter across the square is 65 mm, the diameter of the
usual type of half-pint milk bottles. While the shelf
space is unchanged, the useful area is considerably
larger because of the extra space in the corners. From

the four sides of the bottom the glass rises straight until it is intersected by the cone of the body of the bottle.

This cone of the side walls intersects the bottom at the outer corners, which are slightly rounded, and makes small panels of the lower side walls. This taper from the corners has been quite sufficient to hold the food cake in position solidly. But there is no cylindrical portion of the body to which the food cake could slip if it broke slightly away from the bottom. The straight taper of the sides offers a better surface for the smooth attachment of labels and for writing than did the swelling curve of the milk bottle. Also the cone of the sides makes a smooth funnel to deliver the flies to the etherizer in emptying the cultures.

The present food formula, worked out in cooperation with Helen Redfield and Hugh Darby, is:

Water	75.0 cc
Molasses (free from SO_2)	13.5 cc
Cornmeal (coarse yellow)	10.0 grams
Agar-agar	1.5 grams

About 60 cc of the medium is put in each bottle, giving a cake about 25 mm thick, and seeded with yeast after cooling. While this amount is sufficient to produce 600 to 1,000 flies in stock cultures, it should be clearly understood that it is not enough to give good results for more than five days' egg laying in linkage work, while for best results not more than one day's eggs from one female should be raised in such a bottle.

THE ETHERIZATION OF DROSOPHILAS

During the twenty years that the yeast fly, *Drosophila melanogaster,* has been used in the study of heredity, many millions of individuals have been examined closely. Much time and labor have been spent in the removal of flies from culture bottles and in etherizing them so that they would lie quiet during the separations and recordings. In several respects the early methods of etherization were unsafe as well as slow. To remedy one or an-

other of these difficulties, various types of bottles were tried as etherizers. In 1919 a design was drawn for an etherizer for which the glass part was especially blown. Trial of this model suggested improvements, and the etherizer that is described and diagrammed in this account is the current model with a considerable series of predecessors.

The improvements make the intake of flies from the culture bottles and their discharge to the sorting plate both safe and rapid, the etherization quick and uniform, and the amount of ether used and ultimately liberated into the room very small. The visibility of the flies being etherized or discharged is excellent. The interior of the etherizer and the entrance and exit are easily kept clear and clean. Finally, special means have been employed to insure against breakage in the ordinary course of use or by accidental dropping on a concrete floor.

The entrance to the etherizer (see diagram) is through a metal funnel whose rim fits accurately inside the groove in the mouth of the milk bottles generally used for culture bottles. Between the etherizer and the culture bottle there is no troublesome chink, through which flies might escape. The sheet-brass or galvanized iron of which the funnel is made is so thin (under 1 mm) that its edge offers no ledge that would hold the entering flies from sliding directly on into the body of the etherizer. The slope of the funnel (60°) is steep enough and the bore of the stem (6 mm) is large enough to offer only negligible resistance to the passage of the flies. The interior surface of the stem, which is of block tin or brass, is kept clear and polished by the wooden stopper, which extends completely through the stem and projects a couple of millimeters beyond.

In getting flies into the etherizer from the culture bottle, they are first tapped away from the stopper and then kept away temporarily by turning the base of the bottle slightly upward and toward the light source. The stopper is quickly removed and the rim of the entrance fun-

nel fitted into the groove. The bottle is now turned bottom upward with the etherizer vertically below, and the flies dislodged and caused to rattle down into the etherizer by sharp taps of the bottom of the etherizer on the palm of the hand not occupied in holding the two together. This method is far more rapid than to turn the base of the etherizer toward the light and to depend upon gentle tapping on the bottle to start the flies traveling slightly upward and toward the light. But this method requires that the food be in a firm cake, stuck fast to the bottom of the culture bottle and free of liquid. The new
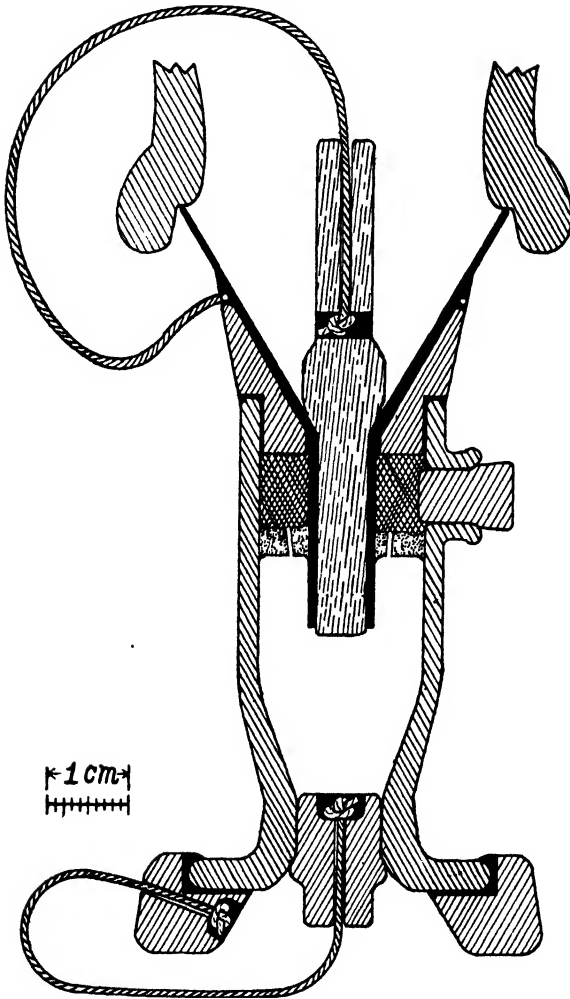
agar-cornmeal-molasses medium, if poured in a cake about an inch thick, is satisfactory. The new conical-sided culture bottles are much better in this respect than the cylindrical-sided milk bottles which were formerly used.

The flies are kept from emerging from the etherizer in a reverse stream by the tip of the stem, which, by extending eight to ten millimeters into the body of etherizer, acts as a trap, making the flies encircle the opening instead of entering and ascending. The entrance is closed, as soon as possible after removal of the etherizer from the mouth of the emptied culture bottle, by a wooden plug. The top of the stopper extends far enough above the rim of the funnel to be easily grasped for extraction. The opposite ends of this top part are flattened to give a good grip. To forestall loss of mislaying of the plug, it is tethered to the neck of the funnel by a short strong cord.

The metal funnel affords protection against breakage of the glass body of the etherizer by being mounted on a cork ring set in the top of the glass. Plastic wood was used to cement the funnel to the cork seat and the seat to the glass body.

The glass walls of the etherizing chamber afford ready observation of the course of the etherization. The chamber is of small volume, which results in quicker initial saturation, less ether and speedier etherization. The relatively small bore of the stem of the entrance funnel, and of the exit, also help to conserve ether, and to maintain the concentration in the interior during and between operations. Besides being economical of ether, the slow escape of ether into the air about the worker reduces discomfort and tends to eliminate ether-colds.

The atmosphere of the etherization chamber is kept at the right concentration of ether by continuous evaporation from a special ether chamber which is separated from the main chamber by a plaster-of-Paris partition. This partition is porous, but to aid the passage of the

ether, about ten small holes were punched through it just before the plaster set. The ether chamber is packed, before pouring the plaster, with asbestos fiber, which holds the ether well and gives a large surface for evaporation. Ether is put into this chamber through a short side-tube closed by a cork. Only about fifteen drops of ether are required initially. Only enough ether should be present to moisten the asbestos fibers and spread over their surface—not enough to leave free liquid or to clog the pores of the asbestos packing. Additions are in even smaller amounts, about five drops, at about half-hour intervals when the etherizer is in use.

When the concentration of the ether is right, the flies are sufficiently etherized in about ten seconds after they are all in the chamber. The ether should not be saturated, nor so concentrated that twenty seconds would be too much. The right duration of etherization depends on the length of time the flies are required to remain anesthetized, which in turn depends on the numbers present, on the number of character differences for which separations are to be made and on the difficulty of the character distinctions. In general, the least etherization that will suffice should be used. In case of very many flies or of difficult and complex separations, it is better to etherize to the standard amount at first, separate the flies into two or four subgroups according to one or two character differences, put these subgroups temporarily into cotton-stoppered vials and then proceed to re-etherize each sublot, for completing the classification and recording. With a few flies, re-etherization can be carried out on the sorting plate, without disturbing the lines of the completed separations, by inverting over the whole group a watch glass with ether on a strip of blotting paper glued to the concave side. A paper handle can be glued to the convex side.

Over-etherization of flies must be guarded against, since it means greater difficulty in manipulation and in classification, and perhaps the loss of flies that ought to

be mated.   Over-etherization is indicated by the posture of the flies, the wings held erect over the back, the legs extended together in a stiff bunch, and the head and abdomen bent toward each other ventrally.

The etherized flies are emptied out of the etherizer through the bottom, after removal of the cork from the center of the bottom.   The walls of the etherizing chamber slope funnel-wise at a convergence of about 30° to this exit.   There is no shoulder to hinder the pouring out of the etherized flies.   Since the exit funnel is of glass, the etherized flies slip easily over its surface.   Also the glass is easy to keep clean, and it is easy to see if a fly should become stuck to the exit funnel.   The seats of the exit cork and of the ether renewal cork are cylindrical, since a cork is gripped better by a straight-sided seat than by one that is flared.   The exit cork, like the wooden plug, is flattened on opposite sides for a grip and is tethered by a cord.

The exit aperture is large enough so that the interior of the etherizing chamber can be easily cleaned by use of wads of cloth or paper held by forceps.

The base of the etherizer is the flared-out tip of the exit funnel and is of thick strong glass.   The base is protected against breakage by being set into a ring of cork, which extends beyond the glass laterally and below. The cement used to attach the cork ring to the glass base is plastic wood.   Plastic wood is also used to cover the countersunk knots of the strings that attach the stoppers to the body of the etherizer.

SHORTER ARTICLES AND DISCUSSION

PARADOXICAL TERMINOLOGY IN GENETICS

THE increasing use by geneticists of the term "recombination" prompts the writer to point out that in the majority of cases the meaning now implied by the geneticist using the word is directly opposite to its true and generally accepted interpretation.

When the two members of any pair of factors segregate in the germ cells and maternal and paternal factors are subsequently brought together in pairs in the zygote, it is quite correct to refer to the latter process as recombination, in the sense that units occurring first in pairs and then separately are finally again combined in pairs. It is therefore correct to say that Mendel's laws depend upon segregation and recombination at random. It was in this sense that the latter term was first used in genetics. However, when one considers the genes introduced in a cross by both parents, it is not correct to refer to all the genotypes or phenotypes obtained in backcross or in the F_2 generation as recombinations, since some of them consist of entirely different combinations of genes and phenotypes from those of either parent, *i.e.*, they are *new combinations*. Paradoxically, it is just these new combinations which are being labelled by many geneticists as "recombinations" when the latter term is really applicable only to the genotypes and phenotypes which are the same as those of either parent.

The error is best illustrated by an example. One may take the coupling phase of a cross in which a new recessive mutation, aa, is being tested for linkage with the dominant character represented by the gene B. If the F_1 be back-crossed to the double recessive homozygote, one obtains the condition listed below.

Parental genotypes;	$AABB \times aabb$
Parental phenotypes:	AB $\quad$ ab
F_1 genotype:	$AaBb$
F_1 gametes:	AB, Ab, aB, ab ·
Phenotypes in back-cross:	AB, Ab, aB, ab

Of these four phenotypes obtained in the back-cross, two, AB and ab, are referred to as "parental combinations." Since they demonstrate the production by the F_1 of gametes containing the

same combination of genes as was contributed in the gametes of either original parent, this terminology is quite correct. On the other hand, the two phenotypes *Ab* and *aB* have not previously been encountered in the cross under consideration. They indicate the production of F_1 gametes containing respectively the gene combinations *Ab* and *aB*. These are strictly new combinations and not "recombinations" in any sense of the word.

When a deficiency of these new combinations below the 50 per cent. expected on the basis of random assortment indicates linkage of *A* and *B*, the gametes *Ab* and *aB* are then referred to as cross-over gametes, or, more commonly, the phenotypes *Ab* and *aB* are simply called cross-overs. However, when there is independent assortment instead of linkage, it is incorrect to refer to the new combinations as cross-overs, since there has been no crossing-over. It has become common usage with some geneticists to label all new combinations as "recombinations" and then to state that their frequency indicates either independence or a certain percentage of crossing over.

Concerning the prefix "re," Webster's Dictionary (1930) says:

A prefix denoting:
1. *Back*, esp. *back to an original or former state or position;* backwards; . . .
2. Again;—used chiefly to form words, esp. verbs of action, denoting in general *repetition* (of the action or of the verb), or *restoration* (to a previous state); as in *rejoin*, to join again, *reiterate*, to iterate again, *renew*, to make new again, . . . etc.

No special meaning is given for "recombination" as is done for numerous other words of which the exact shade of meaning is not clear from the prefix "re" and the root.

With this in mind, whenever no discrimination is made between different combinations of genes, one may say that in a general way all the genotypes and phenotypes obtained in an F_2 or backcross represent recombinations of factors which segregated in the F_1 germ cells. However, when, as in a linkage study, these same genotypes or phenotypes are differentiated into two classes, in one of which the combinations of characters are exactly the same as those found in the parents, while in the other the combinations of characters differ from those of both parents, then, from this aspect, only the former class contains recombinations, whereas the latter class obviously consists of entirely new combinations.

Inspection of the example given above shows that the only recombinations are *AB* and *ab*, *i.e.*, the parental combinations. To be accurate, since "recombination" implies reunion after separation, it is really applicable to the parental combinations only when linkage has not occurred and when there has therefore been in the F$_1$ germ cells independent segregation of the chromosomes bearing the genes *A* or *a* and *B* or *b*. It is not strictly correct to label as recombinations those cases in which *A* and *B* or *a* and *b* are linked and remain together throughout the cross, but the term "parental combinations" is appropriate in cases both of linkage and of independence.

To designate the phenotypes *Ab* and *aB*, which are unequivocally " new combinations," as "recombinations" is not merely making the terminology ambiguous; it is a direct subversion of fact. Geneticists who use the term "recombinations" really mean "new combinations." To them the two expressions seem synonymous, but in actual meanings each is the very antithesis of the other. For this reason, no one but a geneticist could easily understand the paradoxical statement in the second sentence of the following quotation from a leading text-book:[1]

> The two large classes contain the factors in the same combinations in which they occurred in the parents and are, therefore, called parental combinations. The two smaller classes represent combinations of the factors different from those of the parents and are, therefore, called recombinations.

Similar inaccuracy is to be found in many recent papers reporting linkage studies.

Equally undesirable is the use of the term "recombination" in place of "crossing-over," as it is employed in the text just quoted (pp. 136–137) and in other writings on genetics. Crossing-over is a distinct phenomenon in genetics and it is therefore highly desirable to retain for it this accurate and descriptive term by which it is usually designated. Since the process forms new combinations of genes, and not recombinations, it can not with accuracy be referred to as a process of recombination.

In the interest of a comprehensible terminology, the writer suggests that geneticists refrain from using the words "recombinations" and " recombination" in these senses and stick to the terms "new combinations" and "crossing-over." Where the frequency of new combinations indicates linkage, such combina-

[1] E. B. Babcock and R. E. Clausen. "Genetics in Relation to Agriculture." Second ed., p. 126, McGraw-Hill. 1927.

tions may correctly be referred to as cross-overs, but the general term suggested will cover all cases.

Some readers of this note will counter the suggestion with the reply that no up-to-date geneticist who can read English will be confused by the continued use of "recombinations." This may be correct, but it does not exempt us from the mental anathema of the German, Russian or Japanese student who may find it difficult to translate correctly the paradoxical terminology of the American geneticist. After all, since science is exact knowledge, should this knowledge not be expressed in exact and accurate terms?

The intricacies of genetics are not easy to master. The adoption of accurate terminology should make them slightly less recondite to the beginning student, to the foreigner struggling with a strange language and to the worker in other fields who may try to acquire a nodding acquaintanceship with the youngest of biological sciences.

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RESTING EGGS THAT FAIL TO REST

Moina macrocopa is a species of Cladocera found in abundance in barnyard and other similar ponds. Probably most ponds inhabited by this species are formed by the winter and spring rains and frequently dry up completely during the summer. *Moina macrocopa* is a very prolific, though relatively short-lived, animal. Depending on the temperature and food conditions, a female when three to seven days of age will produce parthenogenetically her first brood of ten to twenty young. Thereafter she is likely to release, at thirty- to sixty-hour intervals, successive clutches of fifteen to forty parthenogenetically produced young until she has produced from three to perhaps ten broods. When the pond becomes overcrowded during the summer, due to evaporation of the water as well as the prolificacy of the species, some of the young produced are males and some of the females produce the sexual or resting egg (not more than two per clutch). These resting eggs are laid into the ephippium or modified egg case which is cast when the animal moults. If unfertilized these eggs quickly disintegrate. The fertilized egg is presumably able to withstand the summer drought and the

freezing temperatures of winter and to hatch (into a female) when the pond refills in the spring.

By crowding and control of food supply, the conditions of the wild may be sufficiently approximated in the laboratory that fertilized sexual eggs may be produced experimentally. Agar (1914) and Green (1919) had only moderate success in discovering methods of ending the latent period (inducing the egg to hatch) of sexual eggs produced by *Simocephalus vetulus* (another species of Cladocera which has a life history similar to that described for *Moina macrocopa*). The writer has had fair success with the hatching of ephippial eggs produced by still another cladoceran form, *Daphnia longispina*. All work with Simocephalus and Daphnia indicates that an antecedent latent period of perhaps a week or month or more in length, during which the eggs should be dry, is necessary before these eggs may be induced to hatch. The writer has microscopically examined literally many thousands of *Daphnia longispina* sexual eggs and has never observed a single one developing until after it had become dry and had gone through a resting or latent period. In a discussion of the sexual eggs of Cladocera in general, Storch (1925, pp. 14, 46) says "the resting eggs (Dauereier) immediately after fertilization pass through only the first developmental stages to the mesoderm-analage, when without exception a resting stage sets in which lasts from several days to many months until finally with the occurrence of certain conditions development is continued and carried to its conclusion."

Preliminary work done by Banta (personally communicated) with *Moina macrocopa* sexual eggs indicated that a much shorter latent period before hatching is necessary for this species than for *Daphnia longispina* and that perhaps this period might be as short as twenty-four to forty-eight hours if the eggs were promptly made dry. Consequently *Moina macrocopa* was recently selected to make some genetic tests. The observations here cited on the latent period of the eggs of *M. macrocopa* are a by-product of the other study. In work with *Daphnia longispina* the writer's routine method of handling the eggs has come to include a microscopic examination of the cast ephippial cases a day or more after they were cast in order to get a precise record of the number of fertilized sexual eggs. After such a count, also as a part of the routine, the eggs were removed to a bottle to dry. In the course of the first of such microscopic examina-

tions of sexual eggs of *Moina macrocopa* a quite startling and unexpected observation was made. Two of these eggs were developing and were almost ready to hatch without ever having been dry and not more than forty-eight hours after the egg-case had been cast by the mother. It was regarded as an exceptional occurrence. Several days later, however, another recently laid sexual egg, which had never been dry, was observed to be developing. The method of handling was then altered. After being counted the eggs were transferred to a bottle of fresh culture medium which had stood long enough to be clear. Daily microscopic examinations of the eggs were made. Of one lot of 24 eggs carefully followed in this manner, 20 (83 per cent.) hatched without having been dry. Three which did not hatch after a week of daily examinations were put out in a bottle to become dry. Five weeks later when water was added to the bottle all three hatched. The twenty-fourth egg had been recorded as "probably disintegrating; discarded." From a second group of 60 sexual eggs and from a third group of 67 sexual eggs from the same line, 77 per cent. and 78 per cent., respectively, hatched without having been dry.

It seemed possible that the line of *Moina macrocopa* (Banta's Line 1012) selected for this work might be unique in this presumably extraordinary behavior. Accordingly, an entirely unrelated line of *Moina macrocopa* (Banta's Line 1705), obtained from a pond near Providence, R. I., was tested, the first line tested having been obtained from a pond near Cold Spring Harbor, N. Y. Two groups of eggs were obtained from this Providence stock—one containing 189 and the other 132 sexual eggs. From the first, 88 per cent. and from the second, 86 per cent. hatched without having been dry and within from 2 to 24 days after having been cast in the ephippial case. From still another group of 52 ephippial eggs obtained by mating females of the Providence stock with males of the Cold Spring Harbor stock 83 per cent. hatched without having previously been dry.

As indicated above, these eggs were obtained primarily for another purpose. Only the one lot of 24 eggs was followed in an attempt to ascertain the maximum percentage which might hatch. Twenty-three of the 24, or 96 per cent., hatched. The other lots observed were followed only long enough to obtain sufficient material for the main study in hand at the time. It is probable that somewhat higher percentage of hatches might

have been obtained from these other lots if those eggs which failed to hatch without drying had been dried and had then been placed in water.

Observations have also been made on sexual eggs of a third line of *Moina macrocopa*—Banta's Line 1707—obtained from central Indiana in the fall of 1931. Similar high percentages of prompt hatching of the sexual eggs without a resting period were obtained. Hence this phenomenon of hatching without a latent period occurs in all the stock of this species which the writer has been able to test and may readily be universal for this species.

If these laboratory observations can be considered as having been made under conditions comparable to conditions prevailing in outdoor ponds, the high percentage of early hatches from sexual eggs without a latent period of being dry may be cited as one of the wasteful processes in nature, inasmuch as all individuals hatched or hatching from such eggs would ordinarily die in the course of a few days following their production (during which the pond usually either becomes dry or from other causes fails to continue to sustain this species). In comparison with the number of parthenogenetic young produced, the number of the ephippial eggs produced is very low. These observations indicate that with *Moina macrocopa* most of the sexual eggs develop soon after being laid. Such as hatch have small chance for survival and merely tend to aggravate a situation already unfavorable for the continuance of the active animals. Presumably only the small percentage which fails to hatch without having become dry and such as become stranded and promptly dried along the receding pond margin would remain to carry the race over until favorable conditions again return.

In addition to this adaptation of the sexual egg of Cladocera to carry the species over the vicissitudes of drought and winter, the increased vigor of some of the clones derived by sexual reproduction has been demonstrated (Banta and Wood, 1928). The evidence indicates that this is due, not to a rejuvenescence resulting from sexual reproduction *per se*, but to genetic recombination. There are ponds in which *Moina macrocopa* occurs which do not periodically dry up or freeze over. Further, Storch (1925) states that Leege and H. Spandl have demonstrated the very general transference of sexual eggs from one locality to another on the feathers or feet of water birds. Per-

haps *Moina macrocopa* is generally capable of producing two sorts of sexual eggs—those which require an extended resting period and those which do not.   Regardless, then, of the nature of the body of water—whether or not it dries up or freezes periodically—to which *Moina macrocopa* might be transferred, the species would be able to maintain itself in a vigorous condition.

There is a marked parallelism between this interpretation of the present observations and Bodine's very recent observations (orally communicated) on the development of grasshopper eggs. Bodine and associates find that the same grasshopper may produce two sorts of eggs, one of which ceases its development regardless of temperature (has a resting stage or diapause) and resumes development in the spring after exposure to low temperatures; whereas embryos of the other sort continue their development without a diapause or resting stage.   In temperate regions the former sort survive, the latter kind may perish.   In milder climates either may survive.

I am indebted to Dr. A. M. Banta for his many pertinent suggestions which led to these observations and for his willingness to advise in their presentation.

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COMPLEMENTARY FACTORS FOR EYE COLOR IN DROSOPHILA

BRIDGES ('19) and Bridges and Morgan ('23) have described the effects of a large number of combinations of factors on eye color in Drosophila. They found many cases in which the effects are not simply cumulative. Such cases furnish interesting laboratory material. A considerable number of crosses between different recessive eye colors were made by members of the class in genetics at the University of Chicago in the fall quarter of 1931. Doubtless all have been made before as only familiar mutations were used but as I have not found any published reference to certain of the most striking results, it may not be superfluous to call attention to them. Six members of the class (L. E. Alexander, D. M. Crooks, Mary Talbot, J. A. Miller, Grace Townsend and C. A. Cohn) made the cross between brown eye and scarlet. The red eyed F_1 flies produced an F_2, which in the aggregate included 919 red, 302 scarlet, 327 brown and 89 white, each divided approximately equally into males and females. It appears that scarlet and brown, which individually produce relatively slight, though qualitatively different effect on eye color, produce white as the double recessive. Mr. Crooks and Mr. Alexander tested this conclusion by mating the new white with brown stock, obtaining only brown; with scarlet stock, obtaining only scarlet and with the ordinary sex linked white in which case the daughters (at least) were red eyed.

The effects of the scarlet gene are very nearly if not quite the same as those of the sex linked gene vermilion. The eye colors are indistinguishable unless a tendency of scarlet to darken more with age, noted by Bridges and Morgan, is characteristic. Both have very light ocelli, those of scarlet being described as white and those of vermilion as having a barely detectable tinge of yellow. There is a marked contrast with the brownish red ocelli of red eyed and brown eyed flies. Bridges and Morgan state that the double recessive scarlet vermilion shows no cumulative dilution effect being a vermilion "indistinguishable from both single recessives." The simplest physiological hypothesis for accounting for such cases (of which they give many other examples from eye colors of Drosophila) seems to be that the recessives represent complete inactivation of two genes which are solely responsible each for carrying through a different link in

the same chain reaction. Failure of either link or of both would cause complete absence of the end product of the postulated reaction. In the present case, it must be assumed that this reaction product is not itself necessary for eye pigmentation since its failure leaves the rather intensely colored vermilion (or scarlet) eye.

The absence of pigment in the double recessive scarlet brown indicates that the type allelomorph of brown is solely responsible for an essential link in the complementary pigmentation process implied above. If these deductions are correct both pigmentation processes should also fail in the double recessive vermilion brown, which, therefore, should also be white. This turns out to be the case. Mr. Crooks mated a brown female with a vermilion male. The F_2 from the red eyed F_1 flies consisted of 76 females (54 red: 22 brown) and 79 males (23 red: 7 brown: 34 vermilion: 15 *white*).

The apparently "disproportionate" effect of the double recessive and the qualitative differences between brown and the others present some difficulty but it may be that the two postulated reaction products act primarily on the same otherwise limited process, on which the failure of either has thus only a slight effect but that they differ in effect on a secondary qualitatively different pigmentation process. In this connection, the recognition by Johannsen ('24) of two distinct pigments, wine red and yellow, which varied to some extent independently in the different eye colors, is of interest.

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A CASE OF TWO SIMULTANEOUS MUTATIONS FOR VIRESCENT SEEDLINGS IN MAIZE

THE frequent occurrence of seedling abnormalities in open fertilized maize stocks has been noted by many investigators. There is, however, very little crucial evidence to indicate whether the abnormalities appearing upon selfing are recent mutations or mutations which have been carried along in a heterozygous condition.

The most extensive data dealing with naturally occurring mutations of the chlorophyll deficient type have been presented by Hayes and Brewbaker.[1] They observed four mutations among a total of 953 selfed lines.

In the summer of 1930 the green sibs in a progeny of maize known to be segregating for albinism were tested to determine whether there was any relation between heterozygousness for this character and catalase activity. The tested plants were selfed and classified with respect to albinism. Although differences in catalase activity occurred, there seemed to be no consistent difference between the homozygous green plants and those heterozygous for the albino gene.

Among the ears tested for albino segregations was one which unexpectedly segregated for both albino and virescent seedlings in a ratio of approximately 27 greens to 37 chlorophyll deficients. Such a segregation could be explained on the assumption of contamination by a stock carrying two virescents or the occurrence of two simultaneous mutations for virescent seedlings. Contamination does not offer a satisfactory explanation. No stocks in the field were known to carry two virescent genes. The progeny in which the aberrant ear occurred gave no indication of previous contamination as evidenced by hybrid vigor. Additional evidence against contamination was the difference in vigor between the plants grown from the original stock and those from the aberrant ear. The latter differed from its parent in about the degree to be expected from the additional generation of selfing to which it had been subjected.

The progenies grown from the original ear and from the aberrant ear were selfed to test for freedom from contamination of the one and for the presence of two virescents in the other. In

[1] H. K. Hayes and H. E. Brewbaker, *Jour. Hered.*, 15: 497–502, 1924.

both progenies only the green plants survived. Twenty-three selfed ears were obtained from the stock known to carry albinism. Of these, eight produced progenies of green seedlings only and the remaining 15 produced progenies containing green and albino seedlings in a ratio approximating 3 to 1. There was no evidence of contamination of any of the plants in this progeny. Thirty-five selfed ears were obtained from the progeny of the aberrant ear. The seedling progenies of these ears could be separated into the six classes expected on the basis of segregation for one albino and two virescent factors. The summarized data are presented in Table I.

TABLE I

SUMMARY OF SEGREGATIONS FOR THE CHLOROPHYLL TYPES STATED

	Number		$o-c$	$(o-c)^2$	$\dfrac{(o-c)^2}{c}$
Type of Segregation	Observed	Calculated			
1. All green	2	1.3	.7	.49	.04
2. 3 green : 1 albino . .	5	2.6	2.4	5.76	2.21
3. 3 green : 1 virescent ..	8	5.2	2.8	7.84	1.50
4. 9 green : 7 virescent	7	5.2	1.8	3.24	.62
5. 9 green : 7 virescent and albino	8	10.4	2.4	5.76	.55
6. 27 green : 37 virescent and albino	5	10.4	4.6	21.16	2.03

$$\chi = 6.95 \qquad P = .22$$

Using the data obtained by Hayes and Brewbaker as a standard, a simultaneous mutation for two chlorophyll deficient types would be expected only once in 56,763 trials if one considers all the mutations observed by them or once in 908,209 trials if only the virescent mutation is considered. They emphasize the fact that the mutation rate varies with different varieties and it is of course possible that the stock involved in this case had a very high rate. With the exception of the single aberrant ear there is no evidence to indicate a high mutation rate for this stock.

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EARLY AND LATE FEATHERING IN RHODE ISLAND REDS

Serebrovsky ('22) and Warren ('25) have reported a sex-linked gene for rate of feathering in White Leghorns, Barred Plymouth Rocks, Russian Orloffs and Jersey Black Giants. Warren's data indicate very definitely that late-feathered chicks do not show tail feathers earlier than sixteen days after hatching and that early-feathered chicks exhibit definite tail feathers at nine days of age.

Rapid feathering, which is characteristic of the Leghorn breed, is due to a recessive sex-linked gene called (r) or (sl) by Warren. Since the male fowl carries two X chromosomes and the female but one X, there should be twice as many early-feathering pullets as early-feathering cockerels in a population made up of early and late phenotypes.

The Rhode Island Red flock of the Massachusetts Station hatched in 1931, consisting of 2,882 chicks, was classified into the early and late phenotypes at twelve days of age. These birds have been bred for characters affecting fecundity without regard to rate of feathering. If this is really a mixed population for rate of feathering and if the gene (r) or (sl) is present in this flock there should be twice as many pullets as cockerels in the early feathered class.

RHODE ISLAND REDS HATCHED IN 1931

	Early Feathered		Late Feathered	
	Males (sl sl)	Females (slo)	Males (Sl Sl) or (Slsl)	Females (Slo)
Actual	194	373	1249	1066
Expected	189	378	1247	1068

The sex ratio in the above chicks is very close to equality, there being 1,443 males and 1,439 females when sex was determined at eight weeks of age. There were 567 birds that were classified as early feathering in a total of 2,882. The data actually indicate that in equal numbers of males and females there are twice as many early-feathered females as there are males. The deficiency of males in the early class is almost

exactly made up in the late group and the surplus of early females accounts for the female deficiency in the late class.

The data indicate, therefore, that the sex-linked recessive gene already referred to is present in the Rhode Island Reds studied and that it alone is responsible for the early-feathering observed.

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NOTE ON THE MEDUSA CRASPEDACUSTA IN MISSOURI, WITH A SUMMARY OF THE AMERICAN RECORDS TO DATE

ON September 6, 1931, a single medusa 10 mm. in diameter was found near St. Charles, Missouri, by Michael S. Wepprich, Jr., a student at the University of Missouri. Sections of the gonads showed it to be a male, and Dr. Fernandus Payne, of Indiana University, was kind enough to confirm the identification of the specimen as *Craspedacusta ryderi* (Potts). It was found in a quarry-pit south of the Wabash R. R. right-of-way, in the northwest corner of the southeast quarter of Section 23, St. Charles County. A visit to this place three weeks later failed to locate any more medusae, and the search will be resumed in the spring.

It is apparently agreed that the fresh-water medusae and their ''microhydra'' forms so far reported from various parts of the United States (together with one record from the Panama Canal Zone) should be assigned to the genus *Craspedacusta* Lankester. Whether or not the North American specimens assigned to the European species *C. sowerbii* Lankester should instead have been referred to the American species *C. ryderi* (Potts) has not been decided. Meanwhile, the hydroid of *Craspedacusta* has been found in four different states, and the

medusa in ten states, the District of Columbia, and the Canal Zone. It may be of interest, therefore, to bring together in a table the American records of this uncommon but widely distributed genus which have so far been reported:

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Year reported	Reported by	Locality	Stage and sex	Name given	Publication
1885	Potts	near Philadelphia, Pa.	hydroid	*Microhydra ryderi*	*Sci.*, 5, No. 123, cover sheets, p. v
1897	Potts	*ibid.*	medusa	*M. ryderi*	AMER. NAT., 31, 1032–1035
1907	Hargitt	Washington, D. C.	medusa (male)	*Limnocodium sowerbii*[1]	*Sci.*, 26, 638–639
1916 and 1924	Garman	near Frankfort, Ky.	medusa (male)[2]	*Craspedacusta sowerbyi*	*Sci.*, 44, 858–860 *Sci.*, 60, 477–478
1924	Payne	Elkhart, Ind.	hydroid and medusa (female)	*C. ryderi*	*J. Morph.*, 38, 387–430
1924	Payne	Augusta, Ga.	medusa	not named	*ibid.*
1925	Smith	Gatun Lake, C. Z.	medusa	*C. sowerbii*	*Sci.*, 61, 588–589
1925	Payne	near Frankfort, Ky.	hydroid	*C. ryderi*	*Sci.*, 62, 421
1926	Payne	4 points on Kentucky R.	medusa (male and female)	*C. ryderi* (1 record); other 3 not named	*Biol. Bull.*, 50, 433–443
1927	Schmitt	Potomac R.	medusa	*C. sowerbii*	*Sci.*, 66, 591–593
1927	Schmitt	near Owensboro, Ky.	medusa	*C. sowerbii*	*ibid.*
1928	Breder	New York Aquarium	hydroid and medusa	*C. sowerbii*	*Sci.*, 67, 242
1930	White	Tuscaloosa, Ala.	medusa (male)	*C. ryderi*	*Biol. Bull.*, 59, 222–232
1931	Ortenburger and Phillips	near Broken Bow, Okla.	medusa	*C. ryderi*	*Sci.*, 74, 222
1932	Bennitt	St. Charles, Mo.	medusa (male)	*C. ryderi*	

[1] Species not named; in a later paper (Biol. Bull. 14, p. 312) Hargitt calls these medusae "almost certainly *Limnocodium sowerbii*."

[2] Sex not given in either of Garman's papers; Payne (1926, p. 433, see above) says they were males.

PHYSIOLOGICAL FACTORS NECESSARY TO ALLEVIATE GENETIC LETHAL ANEMIA IN MICE

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THE fact that certain pathological conditions have been shown to be due to definite gene complexes and that these pathological conditions may be eliminated from a strain by the substitution of another equally simple gene complex for the one at fault, suggests that other agents, taken possibly from the environment, could also supplement the unfavorable inheritance to produce a normal individual out of what is potentially a bad inheritance. This paper is based on an inquiry into this problem in the case of a single pathological condition, the lethal anemia of mice, found associated with one gene, the adult character of which is dominant white spotting.

The existence of this gene was first brought to light by Little (1), in 1915, when he indicated that two genes for this dominant white, when present in the same individual, were lethal in their effect. This lethal factor generally kills the animal at or shortly after birth, as both Detlefsen and De Aberle (2, 3) have shown. It is usually difficult to find the young since the mother eats them either because they are obviously weaklings or because they are dead. The young anemics, when obtained alive, are distinctive in appearance. They are generally smaller than the normal individuals of the litter and present a dead white and bloodless appearance. The

anemics often have vigorous appetites and suck large quantities of milk. They are, however, unable to utilize this milk for their proper nutrition due to the presence of this gene, and usually die before the 7th day. If the animal is autopsied the organs, especially those normally having a large amount of blood, are pale in color.

In our investigations, heterozygous matings of the black-eyed white mice carrying the recessive anemic factor produced 103 anemic young mice in 903 births, or 11.4 per cent. Not all these matings were checked every day. In 180 births where the matings were examined morning and night or oftener the number of anemics was 40, or 22.2 per cent.

TABLE I

ANEMIC BIRTHS AND NORMAL BIRTHS OBSERVED IN ALL LITTERS (1),
AND IN THOSE EXAMINED EVERY 24 HOURS OR LESS (2)

	Anemics	Normals	Per cent. anemics of total
(1) All births	103	903	11.4 ± 0.7
(2) Births less than 20 hours old when examined	40	160	22.2 ± 2.1

Under these more controlled conditions the results therefore approach those expected of a simple Mendelian ratio, 25 per cent. Our data consequently conform to those of Dr. De Aberle, in which she showed that for 500 young, 17 per cent. anemics were obtained when the cages were inspected two or three times a day and only 10 per cent. when inspection was delayed for 24 hours or over. Dr. De Aberle further showed that when 16-day-old fetuses were examined *in utero,* the percentage of anemics was 24.6 per cent., practically an exact Mendelian ratio. The fact that the death rate of the fetuses in the uteri of the parents which genetically could produce only normal young was only slightly greater than that for the parents which produced anemics showed that no fetal nutritional factor, fetal congestion or like cause

can be invoked as the primal factor in the production of the anemic progeny.   The fate of these young was sealed at the fertilization of the egg.   The anemia was a matter of heredity.   This conclusion is further borne out by the distribution of anemics in litters of different sizes when the litters were examined every 24 hours.

TABLE II

DISTRIBUTION OF ANEMIC BIRTHS WITH LITTER SIZE

No. of young in litter	No. of Anemics					Total litters
	0	1	2	3	4	
1	1					1
2	6	1				7
3	6	1				7
4	5	6	1			12
5	4	5	2			11
6	3	8	3	1		15
7		2	2			4
8	2	1		2		5
9						
10	1					1
11				1	1	2
Total	28	24	8	4	1	65

The genes for this lethal anemia are an integral part of each daughter cell coming from the fertilized egg. The data obviously suggest the important problem, is it possible to replace artificially these gene effects as manifested by a deficiency of necessary products and, by so doing, enable the animal to react normally in spite of the presence in its cells of these unfavorable genes?   The end somatic reaction produced by these genes is an animal which weighs at birth about 68 per cent. of the normal weight.   The dried weight of the normal mouse is 18 per cent. of the birth weight.   The dried weight of the anemic

mouse is 14 per cent. of the birth weight.   There is consequently relatively less solid in the anemic mouse than in the normal.   The blood has a one third to one fourth of the hemoglobin found in a heterozygous black-eyed white parent and the red cell count is correspondingly reduced.   The heterozygous black-eyed white mice, litter mates of the anemics, have only about three fourths of the hemoglobin found in mice of the same genetic constitution but three months old.   The hemoglobin of the anemic appears to be similar to that found in the normal, according to our colleague, Dr. Anson, who was kind enough to test the carbon monoxide and oxygen bands of the hemoglobin spectra.   Since the red cell count of anemics is reduced to one third to one fourth of that of their normal mates, the anemia appears to be due to lack of red cell formation rather than to an improper proportion of hemoglobin in the cells.

TABLE III

Character	Anemic	Black-eyed white mouse
Birth weight	$0.97 \pm .03$ gms.	$1.39 \pm .05$ gms.
Dried birth weight	$0.13 \pm .01$	$0.25 \pm .02$
Hemoglobin per 100 cc	4.2	12.5
Fe – mg. per mouse	$0.08 \pm .01$	$0.10 \pm .01$
Fe – mg. per gms. of dry weight	$0.56 \pm .07$	$0.43 \pm .08$

The known relation of iron to hemoglobin formation led to the determination of that constituent of the body in the belief that the mechanism of iron metabolism might have been upset by this gene.   The whole mouse was dissolved and the organic matter destroyed by concentrated HNO_3 and H_2SO_4 with a small amount of sodium chlorate added at the end of the digestion to remove the last traces of organic matter.   The residue, when evaporated to dryness, was taken up in 5 cc of weak HCl for analysis by the method of Wong (4).   The

amount of iron was then converted into milligrams of iron per gram of dry weight. The analytical results were irregular, the variation between mice being large. The material shows that the anemic mice contained at least as much iron as the controls when compared on a dry weight basis. This conclusion was to be expected since we are dealing with the newly born animal when the materials found in its body are those directly deposited in it from the mother's circulation. Milk is deficient in iron and most animals which undergo a nursing period have sufficient iron stored in their bodies from their dam to supply the needs of this period. The anemic mouse does not differ from the normal in this regard.

Since anemics are found in the uteri in the Mendelian proportion of 1 anemic fetus to 3 not anemic, it is evident that the principle which prevents the anemia can not pass the placenta from the mother's blood to the anemic's blood, or that the anemic has a substance in its blood which destroys the action of such a principle. If the former is correct the principle would differ from hormones like insulin which are known to pass through the placenta. It would likewise be different from many immune bodies circulating in the blood, since some of these are capable of passing through a placenta of the mouse type to the offspring. The evidence consequently leads to the conclusion that the material which the gene for this anemia has thrown out of balance is incapable of passing through the mother's placenta in amounts adequate for the offspring.

It seemed desirable, in spite of the negative character of the probable result from the use of materials which were capable of passage through membranes or of secretion in the milk, to attempt one other experiment using iron. For this purpose the salt ferric ammonium citrate was used. This material was fed to the mouse, injected into it, and fed to the mother before and after parturition. The material is toxic in large amounts but the animal appeared entirely normal if given a dose somewhat

under this toxic range. The result of these experiments on 12 mice gave no hope of success.

Whipple and his associates (5) have shown that by adding large amounts of liver to an animal's diet it is possible to alleviate both pernicious and secondary anemias. Three vigorous looking anemic mice from two litters were fed ground liver at birth; one was fed rat liver, the other two were fed mouse liver. They died in the usual time for anemic mice, 3 days, 4 days, and 6 days. Another set of mice gave like results. Liver seemed to be of no particular value to this inherited anemia. The liver concentrates might have been more beneficial. However, at this time we had found another method that was successful.

The injection of blood into the peritoneal cavity of the anemic mouse proved successful in maintaining life. There were, however, certain grave difficulties which had to be faced and overcome in accomplishing this purpose. The technical difficulties in obtaining and injecting blood into the mouse are such as to make it difficult to prevent the introduction of injurious contaminating organisms into the peritoneum of the young anemic mouse. And lastly, the abdomen of the anemic mouse is small, delicate, and filled with organs which are easily injured by the introduction of the injecting needle. Considering these difficulties, it is surprising that so many young have been favorably affected by the treatment.

Eighteen anemic mice were injected with blood from normal adult mice. In most cases the blood used for the initial injection came from one of the parents. Five hundredths of a cc was considered to be a large initial dose. The blood for later injections frequently came from unrelated mice. No obvious difference in the reaction occurred when this outside blood was used. Of the 18 mice treated, 11 had a duration of life well beyond that of the untreated anemics. Six had a life span nearly double that of the longest lived anemics. Three mice lived better than 2 months: one 67 days, another 85 days, and a

third 117 days. The average duration of life of 14 anemic untreated controls was 2.4 ± .3 days; that of the 19 treated anemics was 19.4 ± 4.9 days. The frequency distributions of the duration of life of the mice in the two groups is shown in Table IV. It is the extended distribution of life of the treated anemic mice as compared with their untreated controls rather than the average duration of life which is significant.

TABLE IV

DURATION OF LIFE OF THE UNTREATED ANEMIC MICE AND THEIR IMMEDIATE RELATIVES INJECTED WITH BLOOD OF THE NORMAL MOUSE

	Duration of life in days														Average
	1	2	3	4	5	6	7	8	12	13	14	67	85	117	
Untreated anemics	6	3	2	2			1								2.4 ± 0.3
Anemics injected with blood	3	1	1	2	1	1	3		1	2	1	1	1	1	19.4 ± 4.9

The physical development of the anemic mice that survived furnished new information on the action of the gene for this anemia when in the homozygous condition. On the whole, anemic mice at birth are somewhat smaller

FIG. 1. Litter at birth, containing 3 anemics and 1 normal.

than are their litter mates. This may be seen in the
photograph shown in Fig. 1. The degree of this differ-
ence is, according to our weights: anemics $0.97 \pm .03$
grams, litter mates $1.39 \pm .05$ grams. The anemic mice
under normal conditions continually regress in size in
spite of the fact that they suck large quantities of milk
from their dams. The picture is clearly one of the mas-
tery of the gene over the physiological utility of the
nutrition. The normal litter mates grow rapidly from
birth. When the anemics are injected with blood they
commence to put on weight. They never completely over-
come the handicap of reduced size however. Fig. 2

FIG. 2. Litter 7 days of age showing (1) a piebald, (2) a piebald with
heterozygous dominant white spotting and (3) a piebald
with homozygous dominant white spotting.

shows two normal litter mates and an anemic mouse
which has been injected with blood. This anemic mouse
had received a trace of blood the 1st day, and 0.05 cc on
the 3rd. The photograph was taken on the 7th day. The
anemic mouse is obviously smaller than the controls but
at the same time, judged by the growth of the hair, is

nearly as far advanced physiologically. The anemic mice have never overcome the reduced initial size.

The anemic mice open their eyes about 2 days later than their litter mates. The three mice which lived to 67, 85, and 117 days were, respectively, female, male, and male in sex. An attempt to breed these animals together failed. The outcross in both directions likewise failed. Examination of the female showed that her vagina had opened at approximately the normal time, indicating the presence of estrin in the circulation. The animals were all small, delicate and underweight. The males showed but little external development of the testes. On the death of the female the ovaries were found to be very small, the size of those of an immature mouse. The testes of the males were also infantile in size. The histological study of the tissues will appear in a later paper.

The principle, which is altered by the gene for this anemia, may be replaced by the blood of the normal mouse when this blood is injected into the peritoneal cavity, but the anemic mouse is not able to initiate or continue its manufacture at a rate sufficient for the animal's needs and frequently repeated injections of blood are necessary to keep the animal alive.

The three animals which we were able to raise to the age of sexual maturity were inoculated every 2 days for the first week of life and every 3 days for the following week. The animals had then reached an age where it was evident that the type of treatment was able to bridge the pathology produced by the gene. The animals consequently became particularly valuable for possible breeding purposes, etc. Our experience had shown that each injection presented danger of infection or organ damage sufficient to cause death. For this reason the attempt was made to prolong the interval between injections as long as possible. The next injection was delayed for 8 days and the following one for 9 days without apparent ill effects. Nineteen days elapsed before the next injection, the animals being examined daily for symptoms of

insufficiency. The female of the group began to lose weight, her fur became rougher, and her movements sluggish. The animals were then injected, but while the two males showed their usual activity, the female's weight continued to diminish, her fur to roughen, and her actions to be increasingly sluggish. She died 4 days later. The males were injected May 16th and May 23rd at 8 and 7 day intervals following the death of the female. Just before the May 16th injection one of the males was bitten behind the ears by his normal female consort. He was separated from this female but on the 23rd of May had lost more weight and had become weaker. He died shortly after this injection. The last male was injected on May 23rd and 29th, and June 6th, and then no more injections were given for 18 days. By the end of this period his weight had decreased, his appearance was markedly anemic, his fur was rough and he was sluggish. He was then injected with 0.45 cc of blood. Although the animal lived until June 28th, or 4 days after this injection, his appearance remained unchanged.

These results point to the conclusion that after anemic mice reach the age of sexual maturity (we have normal black-eyed white mice bred 28 days from birth) these mice to survive must have a substitute for the principles affected by this hereditary factor. This substitute must be made every 2 weeks or oftener. The depletion of this principle brings the animal, after a time, to a threshold beyond which it can not be revived. It is an obvious suggestion from the character of the anemia that the vital materials affected by these genes and replaced by the blood are the red cells carrying hemoglobin. Other conceivable explanations are possible, however, and in any case we do not know whether it is the introduction of the red cells which act as carriers of hemoglobin or the formed hemoglobin itself which is responsible. Also, in view of the effects on the reproductive cells, it is clear that these genes manifest other reactions for which the introduction of blood does not compensate.

A similar case of replacement of the substance lacking through the developmental effect of a gene complex has been reported by Smith and Mac Dowell (6). Mac Dowell noted that in a silver strain of mice dwarfism appears in ratios suggesting a simple gene difference. This condition appeared suddenly when the mice were about two weeks old. The dwarfs were sterile and showed thyroid, adrenal and reproductive repressions suggesting pituitary insufficiency. Replacement of the deficiency by pituitary transplants resulted in resumed growth, normal thyroids and adrenals and reproduction. The anterior pituitary alone did not show repair. This case is even better than that here described in illustrating how the gene may bring about a deficiency causing a typical pathology which may be alleviated by replacement of the deficiency from the environment.

SUMMARY

The results herein presented show that this anemia may be counteracted by the injection of normal blood which replaces the vital principle or principles necessary to life not found in the presence of this gene. It is thus shown that the gene effect may be replaced by the substitutions of materials taken from without the animal.

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SHAPE CHANGES DURING FRUIT DEVELOP-
MENT IN CUCURBITA AND THEIR
IMPORTANCE IN THE STUDY
OF SHAPE INHERITANCE

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In previous papers (1927, 1930) the writer has pre-
sented evidence that the inheritance of shape differences
in the fruit of *Cucurbita pepo* is governed by a series of
genetic factors differing in the character and intensity
of their effect but clearly amenable to orthodox Men-
delian analysis. He has also shown (1929) that the
major shape differences visible in the mature fruit are
evident in the very early developmental stages of the
flower. Thus a typical "disk" fruit, which is much wider
than long, will have a very similar shape index almost as
soon as the ovary primordium is distinguishable and
indeed when its bulk is no more than one millionth of
that which it ultimately attains at maturity.

A more recent and intensive analysis of developmental
changes in the fruit of Cucurbita, both in pure lines and
in crosses, has abundantly confirmed these conclusions
but has also established the fact that in the progress from
ovary primordium to mature fruit there are certain
minor but perfectly definite changes in shape. These
changes are in many cases distinctive of particular lines,
and are apparently inheritable. Thus there is a general
tendency in most disk types for the ovary and fruit to
become somewhat flatter as they develop, with a progres-
sively greater width (equatorial diameter) in proportion
to length (polar diameter). Such a change, for example,
may be from an index of 1.5 W (one and one half times
as wide as long) at anthesis to 2.0 W at maturity.
Among the more nearly isodiametric types, this change
is also evident but is less marked. In some types the

flattening is continuous from the first, while in others the young primordia at first become slightly more elongated until about the time of anthesis and then grow progressively flatter. Thus the curve of shape index plotted against volume of ovary (and fruit) has a somewhat different character in different pure lines, but in almost all disk and sphere types it rises appreciably between flowering time and the development of large mature fruits; that is, the index increases on the W side, or the structure becomes progressively more flattened, as it increases in size.

The size at which fruit maturity may be reached, however, is markedly variable in *Cucurbita pepo*. When environmental conditions are unfavorable, ovaries may develop into seed-bearing fruits when they have increased but little in size since anthesis; but under more favorable conditions they may enlarge remarkably, sometimes even to a point fifty times as great as the minimum size for maturity. It is significant that regardless of the size attained by a fruit when it stops growing and becomes mature, it retains essentially the shape index characteristic of that particular size on the shape-size developmental curve. Thus a group of plants belonging to a single pure line and essentially homozygous as to fruit shape but growing under diverse environmental conditions may produce mature fruits ranging in weight from one hundred grams to several kilograms, the smaller ones being relatively thick and the larger ones showing a slight but significant progressive increase in degree of flattening.

Furthermore, when two types differing in fruit size are crossed, there is a pronounced increase in the variability of this character in F_2 even though environmental conditions are very similar, presumably owing to the segregation of multiple factors for fruit size; and these genetic differences in size seem to affect the shape index in the same way as do the environmental ones, fruits which are genetically smaller tending to be somewhat

less flat than those which are genetically larger. Thus in a population free from segregation for shape there is a definite positive correlation between fruit size and degree of flattening. It should of course be understood that 'these genetic factors for size are quite different from, and independent of, the genetic factors for shape, as has been shown by the writer for this material (1931), and that the influence which size is found to exert upon shape is merely due to the fact that it modifies the phenotypic expression of a given shape genotype.

The importance of these facts for any study of the inheritance of fruit shape is obvious. In an F_2 population segregating for fruit shape factors, individual plants may differ considerably in the amount of soil nutrients available, in the incidence of fungous or insect attack, or in other respects which affect the vigor of the plant and thus the size to which its fruits will grow before ripening; and in many crosses involving fruit shape the parents also differ somewhat in fruit size, so that there are size differences in F_2 independent of the environment. All these size variations tend to modify the phenotypic expression of the genetic factors for shape and thus to blur the sharpness of their segregation and make genetic analysis much more difficult.

With a view to eliminating these difficulties, at least in part, an attempt was made during the past growing season to determine fundamental fruit shape differences in a few segregating populations by measuring the indices at a relatively early stage in development, when a given arbitrary size had been reached, rather than waiting for fruit maturity. Heretofore it has been customary to take the one or two biggest and best developed mature fruits on a plant (often there are but one or two fruits in all) and to record these as typical for the plant in question. In the case of three F_2 pedigrees, each resulting from a cross of a different disk line with a sphere line and showing clear monofactorial segregation, about 40 plants of each were grown. Of the total which

reached maturity, 92 proved to be disk fruited and 32 to be sphere fruited, a close approach to the theoretical expectation. Instead of determining the index only from a single mature fruit per plant, however, there were harvested and measured from each plant large numbers of ovary primordia and young and partially mature fruits, beginning with primordia only a few millimeters in diameter. For each of these the shape index was found and the volume computed. Enough determinations were made so that for every plant the characteristic shape indices of ovary or young fruit could be ascertained for any size or stage in development, and thus the whole segregating population could be compared, as to the shape index of its members, without the disturbing effect of size differences.

This major advantage could evidently be obtained by using determinations for any arbitrary size up to the maximum of the plant with the smallest fruits, but there were evidently two further advantages to be gained if relatively small ovary primordia rather than larger ovaries or fruits were chosen for shape analysis.

First, there is evident even in the same plant and at the same size and stage of development a certain amount of fluctuating variability of ovary shape, apparently due to differences in the conditions under which the primordium began its growth. These differences persist to later stages and make it advisable, even in the case of mature fruits, to average the shape indices of as large a number as possible. Such fruits are necessarily few on a single plant, but of young primordia the number available is very large since more are continually produced as earlier ones are harvested; and by the use of these larger numbers a more reliable and typical shape index for the plant can be determined.

Second, the earlier primordia are less affected by factors which control the course of later development. For each plant in these three pedigrees the curve of ovary and fruit shape index on volume could be rather accu-

rately determined, and a few typical curves for disk
fruited F_2 plants are shown in Fig. 1. Some of these are
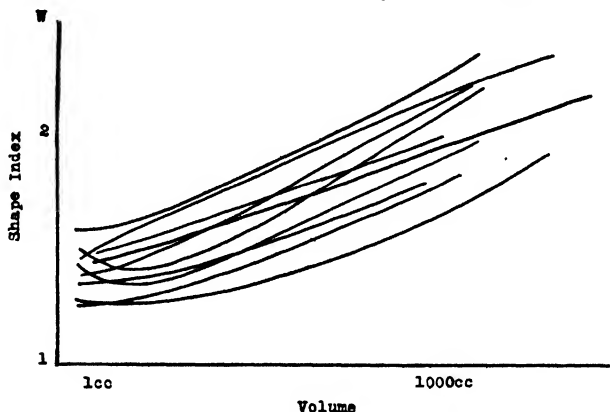
FIG. 1. Changes of shape index of ovary and fruit with increasing size, in
ten representative F_2 disk-fruited plants of pedigree 631.

clearly steeper than others and the shape of the curves
also varies, with the result that there is a considerably
greater divergence in shape at a volume of 1,000 cc than
at 1 cc. The cause of these differences probably lies in
the segregation of minor genetic factors controlling
growth and development, but it is evident that these ex-
ercise their influence relatively late.

To determine whether these presumptive advantages
were actually existent, analyses were made of ovary
shape in these three F_2 pedigrees at various stages.
Data were especially abundant, naturally, for the small
sizes and especially for the class with volumes between
1 and 8 cc (1^3 to 2^3). These primordia are about half the
size of the ovary at anthesis. For each plant the shape
indices of all primordia between the volumes of 1 and 8
cc were averaged and this value taken as the index for
the plant. Then the index of the largest mature fruit
(or the average for several, if available) was determined,
as had ordinarily been the practice. These indices were
plotted, the more elongate types to the left and the flatter
types to the right of the isodiametric point.

The frequency polygons for these three segregating F_2
populations, both of the primordia and of the mature

fruits, are shown in Figs. 2, 3, and 4. In every case there
is, of course, a considerable shift of the indices toward
the right (flatter side) between primordia and mature
fruits. The most notable feature of these figures, how-
ever, is the much sharper segregation and more sym-
metrical distribution of the indices of the primordia. In
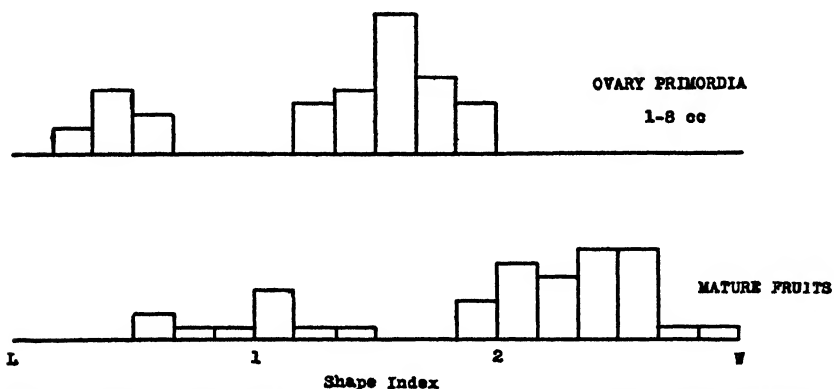
FIG. 2. Shape indices of ovary primordia 1–8 cc in volume (above) and of
mature fruits (below) in F$_2$ 831.
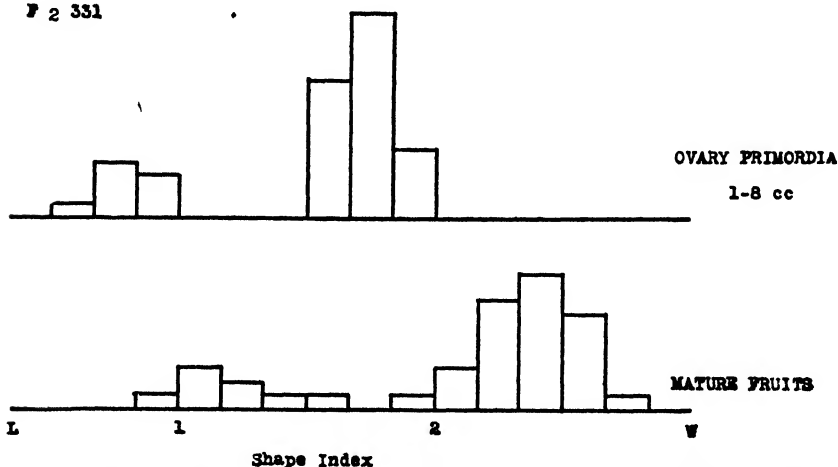
FIG. 3. Shape indices of ovary primordia 1–8 cc in volume (above) and of
mature fruits (below) in F$_2$ 331.

F$_2$ 831, both primordia and mature fruits show clear seg-
regation into two groups, but in the latter these are closer
together and each covers a wider range. In F$_2$ 331, the
two groups of primordia are also widely separated but

they approach closely in the mature fruits, on account of the increased spread of each. F_2 631 is particularly significant. Here the disk parent was much less flat than in the other two pedigrees and thus was closer in index to the sphere parent. Nevertheless, in the primordia the two segregating groups are clearly distinct, whereas in the mature fruits they have merged into a bimodal population. Here segregation is obviously taking place, but between the two modes occur individuals which on the basis of an analysis of the mature fruits alone could not be definitely assigned to either the disk or the sphere segregates. Knowing the shape of the primordia on
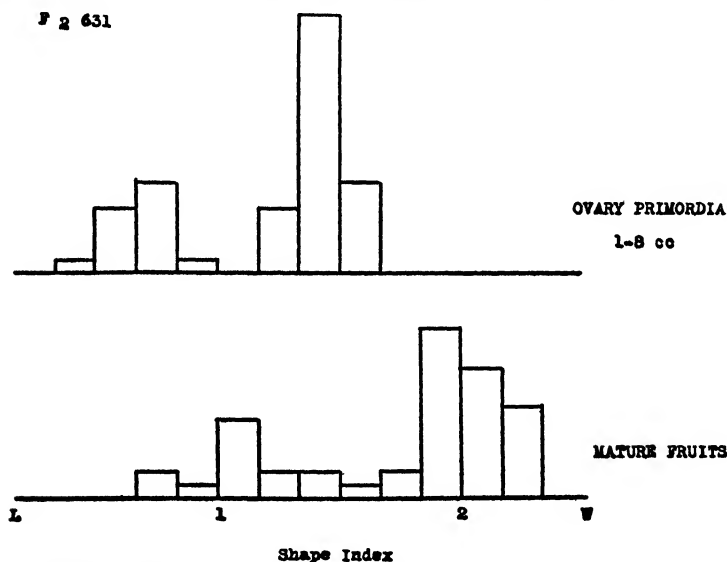
FIG. 4. Shape indices of ovary primordia 1–8 cc in volume (above) and of mature fruits (below) in F_2 631.

these plants, however, we could determine definitely which were genetically disk and which genetically sphere.

In these pedigrees segregation is obvious and genetic analysis easy from a study of the mature fruits alone, since the case is obviously a monofactorial one with dominance complete; but in more complex crosses, where the segregating groups are more difficult to separate, it is to be expected that a study of the ovary primordia will make the segregation more sharply visible and the

genetic analysis much more certain than as if mature fruits alone were investigated. Preliminary studies indicate that this new method will be fruitful in such complex populations.

The present study also indicates the existence, at least in this material, of two types of shape factors, perhaps not differing in kind but certainly in degree: major ones, which operate from the very beginning of development and almost from the start divide the plants into sharply different types, in the present case into disks and spheres; and minor ones, which exert their chief effect later, in modifying slightly the direction and rate of shape change during development.

Summary

(1) During fruit development from early ovary primordium to maturity, in disk fruited and sphere fruited plants of *Cucurbita pepo,* there is a slight progressive increase in degree of flattening, or ratio of equatorial diameter to polar diameter.

(2) Fruits will stop growth and become mature at widely different sizes if environmental conditions vary or if there is segregation for size factors. The mature fruit retains the shape index characteristic of the developmental stage which it had attained when growth ceased. Thus the segregation of genetic factors controlling shape is rendered much less distinct if there are size differences in the population.

(3) By determining shape indices for small ovary primordia instead of for mature fruit, a population segregating for shape may be studied at a uniform size and the variability due to size differences may be eliminated. This method has the added advantages of making possible a much larger number of determinations for each plant, and of eliminating minor shape differences which often appear during development.

(4) In three F_2 pedigrees showing monofactorial segregation for disk and sphere fruit shape, a comparison

of the plotted indices of young primordia, 1 to 8 cc in volume, with those of mature fruits from the same plant showed in every case a much sharper segregation for shape in the primordia than in the fruits.

(5) It is believed that this method of studying earlier stages in development will make possible a more accurate analysis of shape inheritance in cases where the genetic situation is more complex.

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A STRUCTURAL CHANGE IN THE CHROMO-SOMES OF MAIZE LEADING TO CHAIN FORMATION[1, 2]

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AMONG the numerous strains of maize recently assembled for study at Madison one obtained from Manchuria through the U. S. Department of Agriculture was found to be segregating for partial sterility. Crosses between partially sterile plants of the Manchurian race, called M–sterile, and our standard, or o–normal line, give a certain proportion of incompletely fertile offspring. This behavior suggests that a structural change of a compensating character has occurred in the chromosomes of the foreign line giving rise to the M–sterile plants.

AMOUNT OF ABORTED POLLEN

Counts on 38 partially sterile segregates from the cross, normal ♀ × M–sterile ♂, show a mean percentage of defective pollen grains of 23.9. The variation in amount of abortion, however, is rather high, as indicated by a standard deviation of 4.83 per cent. One plant in the population produced 39.2 per cent. empty grains, and two plants gave a little less than 15 per cent. But aside from these three possibly aberrant cases, the variates appear to form a homogeneous group around the mean value. Slightly less than one quarter of the pollen grains formed by typical M–sterile plants are obviously non-functional. While counts have not been made, the partially filled condition of the ears borne by these indi-

viduals indicates that a corresponding proportion of female gametophytes fails to develop. As will be discussed later, certain types of matings involving M–steriles give rise to individuals with much higher amounts of aborted pollen. These plants comprise a distinct class, however, and are termed "high steriles."

Evidence for a Simple Translocation

Cytological examination of the pollen mother-cells of several M–steriles shows that at diakinesis they regularly form eight bivalents and a group of four chromosomes arranged in an open chain (Fig. 1). In normal maize,
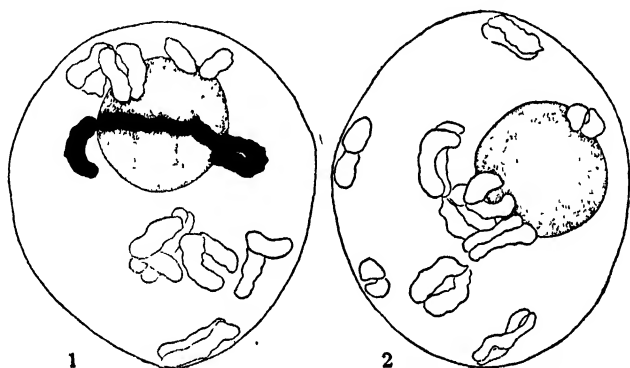
Fig. 1. Nucleus of microspore mother-cell of the M-sterile strain of corn at diakinesis showing 8 bivalents and a chain of four chromosomes.
Fig. 2. Same of normal maize showing ten bivalents.

on the other hand, ten bivalents are found at this stage (Fig. 2).

The occurrence of a chain of four chromosomes at diakinesis and the production of about 25 per cent. aborted pollen suggests that in the M–sterile race a simple translocation has taken place in which a terminal segment of one chromosome has been displaced and reattached by its broken end to the end of a non-homologous member of the complement. The postulated change in structure is illustrated diagrammatically in Fig. 3. If there is association of homologous ends at diakinesis it would be expected that the two modified chromosomes and their normal mates would be united in chain fashion.
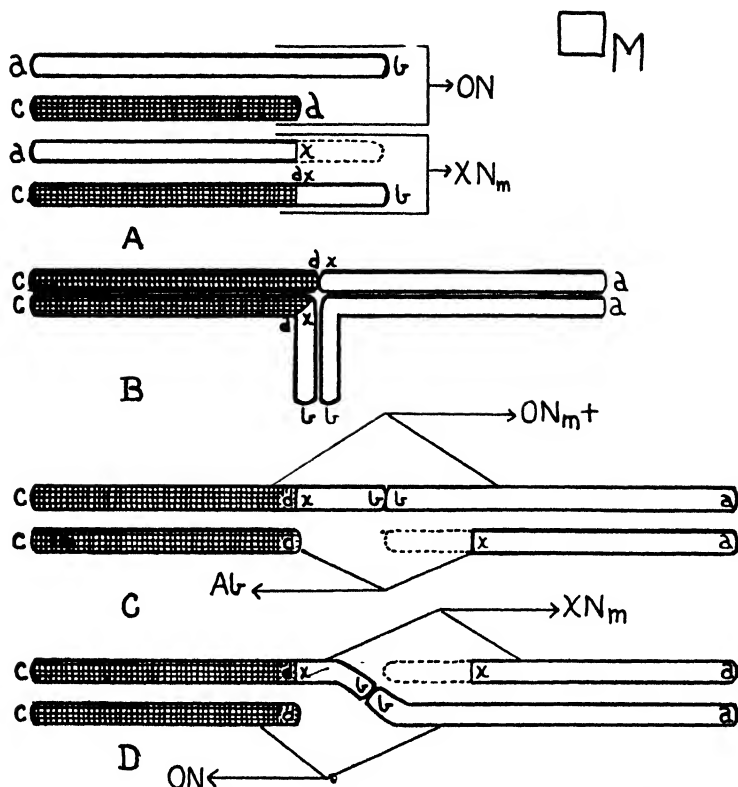
FIG. 3. Diagrammatic representation of the postulated change in structure bringing about the chain of four chromosomes in M-sterile plants. *A*. The two types of gametes; *B*. Type of figure to be found in the open spireme stage. Chains oriented on the spindle so that C, end chromosomes pass to the same pole and D, alternate chromosomes pass to the same pole.

If the group arranges itself on the heterotypic spindle in such a way that the deficient chromosome and its unaltered mate always pass to opposite poles and that the other pair assorts at random with reference to them four types of spores would be formed in equal numbers. As shown in Fig. 3, *D*, the one class of spores will receive the two unmodified chromosomes (o–normal); a second class will obtain the deficient chromosome together with the member of the other pair which has the translocated segment attached. These spores are potentially M–sterile producers and are termed x–normal. A third class of spores, as illustrated in Fig. 3, *C*, will receive a complement carrying the translocated piece in duplicate

(o–n +); and the fourth type will be deficient for the
displaced segment. It might be expected that the first
three kinds of spores would be capable of developing
gametophytes and that the last class would abort because
it lacks entirely the genes carried by the translocated
piece. On this hypothesis 25 per cent. aborted pollen is
called for. It will be recalled that, on the average, 23.9
per cent. was found.

At metaphase in the pollen mother-cells of M–sterile
plants two types of distribution of the chromosomes in
the chain are commonly observed. In the first, as illus-
trated in Figs. 4 and 5, alternate chromosomes pass to

FIGS. 4 and 5. Equatorial plates wherein the alternate chromosomes of the
chain pass to the same pole. FIG. 6. Equatorial plate showing the
end chromosomes of the chain passing to the same pole.

the same pole. It will be seen from diagram *D* in Fig. 3
that this should result in o–n and x–n spores. In the
second type of distribution, as shown in Fig. 6, the end
members of the chain pass to one pole and the other two
to the opposite pole. This mode of distribution pre-
sumably leads to the production of equal numbers of
o–n + spores and spores deficient for the translocated
segment (Fig. 3, *C*). Since about 25 per cent. of the
pollen is aborted, it may be assumed that the two types
of orientation on the heterotypic spindle of the chromo-
somes in the chain occur with approximately equal
frequency.

In 40 figures analyzed from four M–sterile plants, two
cases were found in which the assortment was different
from the above. The end members of the chain were evi-
dently passing to opposite poles, and the other two

chromosomes were either failing to disjoin at all or were so oriented that if disjunction took place each daughter nucleus would receive two adjacent chromosomes. These types of distribution would lead to four aberrant kinds of spores, three of which would be deficient in chromatin material and would probably abort.

Breeding Behavior

The breeding facts relating to the M–sterile race are summarized in Table 1. When simple chain-formers are self-pollinated about two thirds of the offspring are partially sterile and approximately one third are normal. Two families of this type were studied containing 35 and 39 plants, respectively. In the first group 68.5 per cent. of the plants were partially sterile and in the second 69.2 per cent. In matings of the type, M–sterile ♀ × normal ♂, a ratio of two partially steriles to one normal is also obtained. The one family examined gave 30 partially steriles and 16 normals. The deviation from a 2:1 ratio in this case is very small. On the other hand, the odds against the distribution being a chance deviation from a 1:1 ratio are about 32 to 1. The reciprocal cross, normal ♀ × M–sterile ♂, gives a different result. Eighteen families from matings of this kind showed 145 partially steriles and 154 normals, a relatively close approxi-

TABLE 1

The Behavior of the M-sterile Complex in Inheritance

Type of mating		Number of offspring	
		Partially sterile	Normal
M-sterile, selfed	Observed	51	23
	Expected (2:1)	49	25
M-sterile, ♀ × Normal, ♂	Observed	30	16
	Expected (2:1)	31	15
Normal, ♀ × M-sterile, ♂	Observed	145	154
	Expected (1:1)	149.5	149.5

mation to a 1:1 ratio. The numbers in the breeding experiments are rather small, but the results appear to be of an orderly character.

Taken in conjunction with the cytological findings and the amounts of aborted pollen in the partially sterile segregates the breeding facts may be interpreted in the following way. M–sterile plants produce three equally frequent kinds of potentially functional spores, o–n, x–n and o–n +. In the latter class the translocated segment is present in duplicate. All three types give rise to eggs. It is assumed, however, that the male gametophytes which are disomic for the translocated segment (o–n +) are markedly retarded in development and rarely participate in reproduction. Following self-pollination, accordingly, three kinds of eggs are fertilized by two kinds of sperms, as illustrated in Fig. 7.

Two kinds of normal zygotes will result, o–n and x–n, through fertilization of eggs of these respective classes by sperms of like kind. These two types of plants are phenotypically indistinguishable from each other presumably, but are marked off from the other classes by the production of all sound pollen. Two of the six combinations shown in the diagram are the familiar chain-forming M–steriles. This class is easily recognized by its having about one quarter aborted pollen. Approximately half of the partially sterile offspring of selfed M–steriles, however, give amounts of aborted pollen significantly in excess of the 24 per cent. typical of chain-formers. Six plants of this sort upon which counts were made averaged 51 per cent. empty pollen grains. It was suspected that these "high steriles" represented the two classes of zygotes indicated in the right-hand column in Fig. 7. Such plants are hyperploids, being trisomic for the translocated segments.

HIGH STERILES

Cytological study of two high steriles has afforded evidence that this class of plants is actually trisomic for
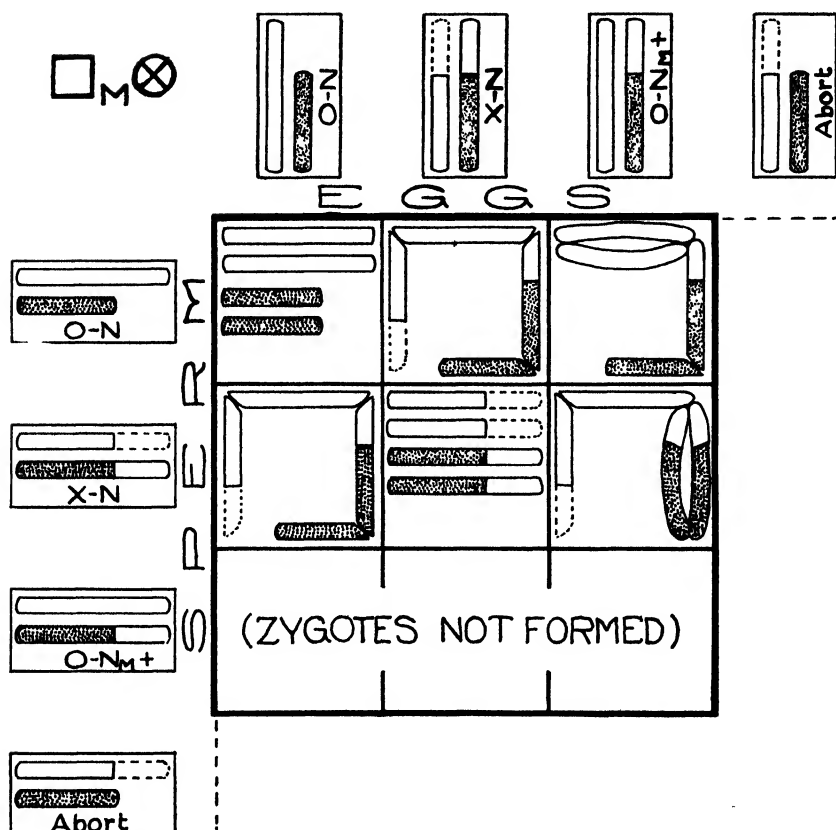
FIG. 7. Diagrammatic representation of the types of zygotes formed as the result of selfing an M-sterile plant.

the displaced chromosome piece. An early diakinesis stage in one of the high steriles is illustrated in Fig. 8. In the three-pronged chain complex, shown in solid black, two of the arms are clearly made up of two strands, whereas the third arm is composed of three. Fig. 9 shows the group separated into its component parts. Both members of the longer pair of chromosomes have a conspicuous lump near one end. The shorter pair consists of chromosomes unequal in length. This difference is due to the fact that the longer of the two chromosomes possesses the aforementioned segment containing the lump while the shorter member lacks it. Evidently the part showing the lump is the translocated piece and the plant is trisomic for it. In Fig. 10 a similar chromosome
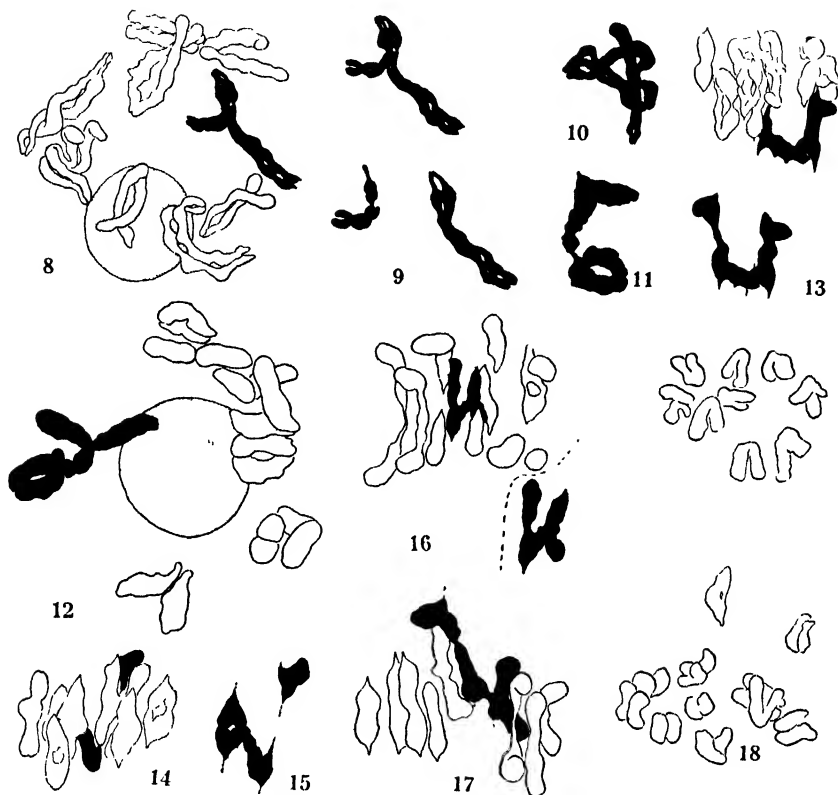
FIG. 8.   Chromosomes of a pollen mother-cell of a high sterile plant at an early stage of diakinesis showing the chain complex and 8 bivalents. FIG. 9.   The chain complex of figure 8 separated into its components parts. FIG. 10.   A similar chain complex from another stage in diakinesis.   FIGS. 11 and 12.   Late diakinesis.   FIGS. 13 to 17.   The behavior of the chromosome complex on the heterotypic spindle.   FIG. 18.   Late anaphase showing 9 chromosomes at one pole and 11 at the other.

complex from the same plant is shown.   In late diakinesis structures like those shown in Figs. 11 and 12 are found. In the latter figure it will be noted that the ends of three chromosomes are associated.   In Figs. 13 to 17 the behavior of the chromosome complex on the heterotypic spindle is shown.   Apparently non-disjunction occurs in a large proportion of the cases, since figures like number 18, showing nine chromosomes at one pole and 11 at the other, are abundantly found in the high steriles.   The higher percentage of aborted pollen in this class of plants doubtless follows from the non-disjunction.

Hyperploids may arise from self-pollinated M–steriles in two ways: an o–n + egg may be fertilized with an o–n or an x–n sperm. In their gross features the chromosome configurations resulting should be similar in the two cases (Fig. 7).

The amounts of aborted pollen in the partially sterile segregates from reciprocal crosses between normals and M–steriles should afford a test of the non-transmissibility, through the male gametophyte, of gametes carrying the translocated segment in duplicate. As stated in the second paragraph, only one plant in 38 partially steriles from the cross normal ♀ × M–sterile ♂ showed an amount of aborted pollen (38 per cent.) approaching that found in high steriles. In the case of the reciprocal cross, M–sterile ♀ × normal ♂, on the other hand, 14 of the 30 partially sterile segregates gave over 40 per cent. aborted pollen. Possibly o–n + gametes do function occasionally through the pollen. The plant producing 38 per cent. aborted pollen mentioned above may have arisen in this way. Material was not available, however, from which the chromosome make-up of this individual could be observed.

Little can be said at present regarding which two of the ten pairs of chromosomes in maize are involved in the M–sterile translocation. It is expected that data from linkage tests will be available next summer. Cytological examination of hybrids between x–normal–1 and M–steriles show, however, that the semi-sterile–1 ring is independent of the M–sterile chain (Figs. 19 and 20). This rules out the *B–lg* and *P–br* chromosomes (Brink and Cooper, 1931). In hybrids with $xn_{1.2}$ (homozygous for the segmental interchanges found in both semi-steriles–1 and –2), on the other hand, the M–sterile chain is associated in a group of six chromosomes, an additional ring of four chromosomes being independent (Figs. 21 and 22). It appears, therefore, that the semi-sterile–2 ring and the M–sterile chain possess one pair of chromosomes in common. As Burnham (1930) has shown, one of the
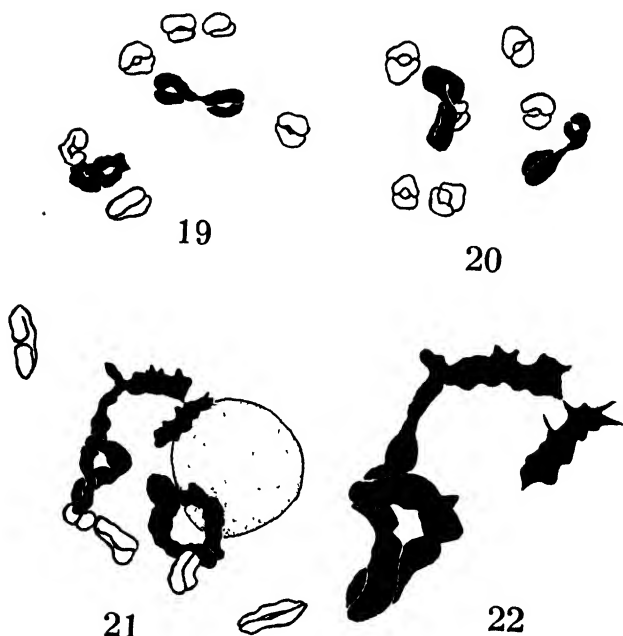
Figs. 19 and 20. Chromosomes of the hybrid x-normal-1 and M-sterile showing that the semi-sterile-1 ring is independent of the M-sterile chain. Fig. 21. Chromosomes of a hybrid between $xn_{1,2}$ and M-sterile showing 5 bivalents, a ring of four chromosomes and a complex involving six chromosomes. Fig. 22. The chromosome complex of six chromosomes. Much enlarged.

chromosomes involved in the semi-sterile–2 ring carries the *c–sh–wx* (aleurone color—shrunken endosperm—waxy endosperm) loci. Burnham also has unpublished evidence, which is cited with his approval, that japonica shows linkage with semi-sterile–2. The position of the japonica gene, however, has not been determined. It may lie within the tenth linkage group, which is not yet definitely established.

Through cytological examination of the high sterile segregates from the cross, M–sterile ♀ × o–normal ♂, it should be possible to distinguish between the donor and the receiver chromosomes in the chain. Following this type of mating all the high steriles will be of the o–n + type, as illustrated in the upper right-hand cell in Fig. 7. The two identical terminal units in the group are normal representatives of the donor chromosome. Material of

this sort at a suitable stage for cytological study, however, is not now at hand, so that the question as to which is the donor chromosome must be left open for the present.

CRITICAL CYTOLOGICAL EVIDENCE FOR TRANSLOCATION

If the behavior of the M–sterile race is the result of the translocation of a terminal segment of one chromosome to the end of a non-homologue it should be possible to obtain critical cytological evidence of the structural change in the early prophase stages of the pollen mother-cell. If the translocated piece is attached in its new
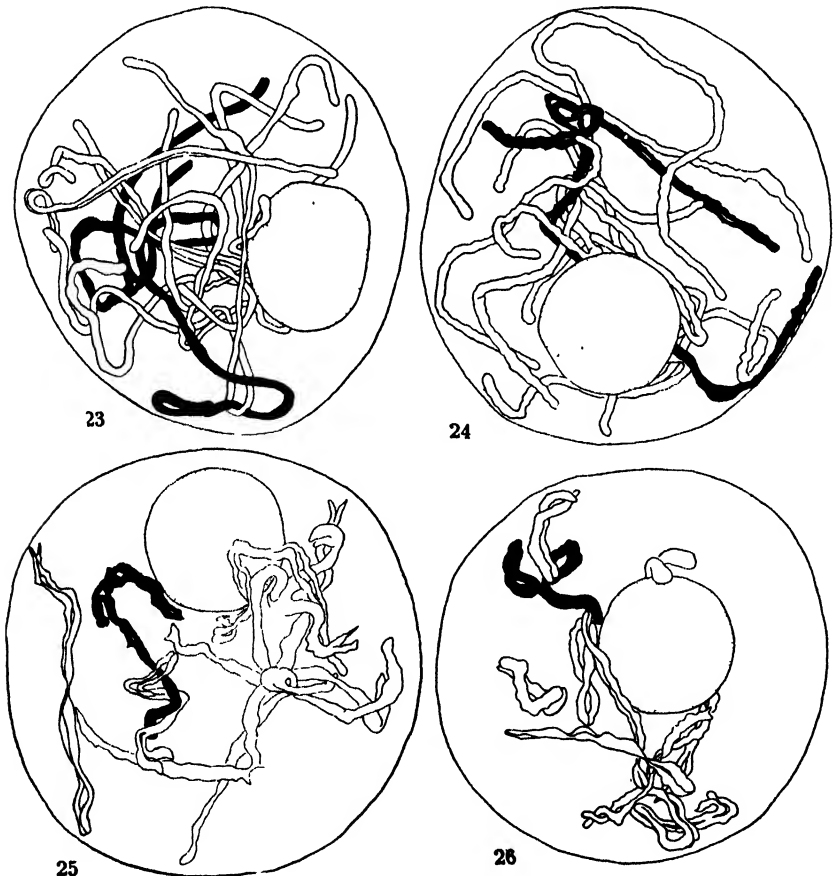
FIG. 23. Early spireme stage of a microspore mother-cell of an M-sterile plant showing a three-armed chromosome complex. FIG. 24. An open spireme stage. FIG. 25. A somewhat later stage. FIG. 26. Early diakinesis.

position by its broken end, and homologous parts of the two pairs of chromosomes synapse, a three-armed structure should result. A detailed study was made of the early stages of a typical M–sterile plant which produced 25.5 per cent. aborted pollen. Some of the configurations found are illustrated in Figs. 23–26. In the comparatively early spireme stages represented in Figs. 23 and 24 a three-armed chromosome complex, shown in solid black, is seen. Figs. 25 and 26 are based on older pollen mother-cells. Appreciable contraction has taken place, but the three-armed structure which will later form the chain is still evident. These cytological observations provide further crucial evidence that the M–sterile line is characterized by a simple translocation.

SUMMARY

(1) The M–sterile race of maize studied shows eight bivalents and a chain of four chromosomes at diakinesis. About 24 per cent. of the pollen grains abort and the ears are incompletely filled.

(2) M–steriles selfed give partially sterile and normal offspring in the ratio of 2:1. The cross M–sterile ♀ × normal ♂ gives a like result. The reciprocal combination, normal ♀ × M–sterile ♂, however, produces equal numbers of partially sterile and normal offspring.

(3) When M–steriles are used as pistillate parents about half the partially sterile offspring give amounts of aborted pollen considerably in excess of 24 per cent. These plants are termed "high steriles."

(4) It is assumed that in M–sterile plants a terminal segment of one chromosome has become detached from its normal position and affixed by its broken end to a non-homologous whole chromosome.

(5) The three-armed chromosome structure expected on this hypothesis in the heterotypic prophase has been identified and is illustrated.

(6) At the heterotypic metaphase, commonly, either the end members of the chromosome chain pass to one

pole and the other two to the opposite pole or alternate chromosomes go to the same pole.

(7) Of the four resulting types of spores one is deficient for the translocated segment and aborts, one carries a normal chromosome complement and one the two chromosomes modified by the translocation. The fourth type carries the "receiver" chromosome with the displaced piece attached and a normal "donor" chromosome; it is disomic for the translocated segment.

(8) The difference in reciprocal crosses is due to the non-transmission of gametes disomic for the translocated segment through the male gametophytes.

(9) Cytological evidence is presented showing that the "high steriles" are hyperploids. The trisomic condition of the translocated segment leads to frequent nondisjunction.

(10) The M–sterile chain possesses one pair of chromosomes in common with the semi-sterile–2 ring.

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METHODS FOR DISTINGUISHING BETWEEN DUPLICATIONS AND SPECIFIC SUPPRESSORS

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SEVERAL cases in which the principal effect was the suppression of a particular mutant character in *Drosophila melanogaster* have been reviewed or described in a preliminary way by one of us (Bridges; in press, *Quarterly Review of Biology*.) Some of these suppressions were clearly due to the presence of a duplicating fragment of chromosome, whose suppressing action was through the wild-type allelomorph carried in the duplication. Others were attributed to the action of specific inhibitors which were mutations in a single gene different from and located far from the gene for the character suppressed. Some of the cases at first explained on the hypothesis of duplications have now been proved monogenic mutations instead. In this paper we wish to review briefly the older methods for determining whether a particular suppression belongs to the duplications or to the specific suppressor category, and to present a new method for distinguishing conclusively between these hypotheses.

That the suppressor is a translocated wild-type allelomorph rather than a mutation in another gene has been proved in certain cases by cytological demonstrations of the duplicating sections of chromosome. Even when the duplication is too small for cytological demonstration the genetic evidence may be strong enough to be conclusive. This was true for the suppressions of morula, speck, balloon and other mutants at the right end of the second chromosome through action of PIII-duplication (Bridges, '19). That the suppression is not due to a duplication of the wild-type allelomorph is clear when the suppression arises in a stock homozygous for the gene

suppressed. But even then the suppressor might still be due to duplication of the recessive mutant gene, with summation effects of the kind assumed in Stern's ('29) explanation of certain bobbed suppressions.

That a given case of suppression does not belong to the duplication category had been fairly certain in several instances, but had not been rigorously proved in any of them. In only two instances had a method capable of giving a decisive answer been applied, but in each case with inconclusive results.

The first of these instances was concerned with vermilion-suppression, which was at first interpreted as a duplication; in fact, the hypothesis of sectional-duplication was invented to account for the phenomena encountered there (Bridges, '19). Among other things, the absence of the expected diminution of crossing-over in the neighborhood of the suppressor and the fact that the suppression acted for vermilion and for sable, ten units apart, but did not act for the mutants miniature, dusky and furrowed between them, had raised serious doubts of the validity of the duplication hypothesis as an explanation of this case. A decisive test was sought in the interaction of "vermilion-deficiency" with the suppressor. If the vermilion-suppressor were really a duplicating wild-type allelomorph, then the combination $v/v\text{-def}/+^v$ should be roughly equivalent to the normal heterozygote, $v/+^v$, and hence be wild-type. But it was found (cultures 6927, 6945, Table VII, Bridges, '19b) that females carrying vermilion-deficiency in one X and in the other both vermilion and the suppressor, showed vermilion eye color and not the wild-type red of suppressed vermilion. Unfortunately for the certainty of the conclusion that the suppressor was not a duplicating wild-type allelomorph but was a recessive mutant with its proper locus near the left end of the X, was the doubt that the vermilion-deficiency was itself genuine. None of the diagnostic points of vermilion-deficiency were strictly unassailable, so that it remained only more highly proba-

ble that the vermilion suppressor was a recessive gene mutation rather than a duplication.

Bonnier ('27) suggested a test by means of the wild-type allelomorph present in the triploid or 3N type. He assumed that if the vermilion suppressor is a recessive gene near the left end of the X, two doses of this recessive should be ineffective in competition with a single wild-type allelomorph. This expectation was in conformity with the general rule, to which the anomalous behavior of plexus with P_{III}-duplication was the only striking exception. A vermilion triploid (v/v/v) carrying in addition two suppressors ($s^v/s^v/+^{sv}$) should not give suppressing action, and hence the eye color should be vermilion. But if the suppressor is a duplicating wild-type allelomorph, then the eye color should be red ($+^v v/+^v v/v$). Unfortunately, the single vermilion female which Bonnier secured and assumed to be of the required constitution turned out later to have carried only one representative of the suppressor instead of the necessary two. The vermilion eye-color of this 3N female was therefore not diagnostic. The red-eyed diploid offspring of this female were all equationals, and the percentage in which they occurred (7 per cent. of the diploid daughters) agrees well enough with the 11 per cent. found (Bridges and Anderson, '25) for genes at the left end of the X. Hence, although the triploid test suggested by Bonnier is theoretically sound, the nature of the vermilion suppressor was left still undecided.

For conclusive evidence we have made use of one of the duplicating fragments (Duplication 101) kindly supplied to us by Dr. Dobzhansky and obtained by him in x-ray experiments (unpublished). This duplication covers the loci yellow, scute and silver at the left end of the X and, at the right end, includes bobbed and the spindle-fiber. This fragment is cytologically demonstrable, and constitutes therefore a real duplication for the mutants suppressed. Males carrying the fragment in addition to one normal X are normal in fertility and in most of their somatic characteristics. Since the locus of the vermilion

suppressor is very close indeed to that of yellow there was a high probability that Duplication-101 would be found to include this locus also. Accordingly, females homozygous for vermilion and for the suppressor of vermilion were mated to males carrying the duplication.

TABLE 1

Suppressed vermilion ♀ [su-v v/su-v v] × Dup-101 ♂ [y sc/101]

March, 1931	Wild-type ♀	+ ♂ [su-v v]	v ♂ [su-v v/101]
3,189	81	43	44
3,190	57	14	44
18,879	97	37	55
Total	235	94	143

In the F_1 progeny (Table 1) all the sons carried vermilion and its suppressor, while approximately half of the sons got the duplication from their father in addition. As the table shows, about half of the sons were red-eyed, having the vermilion suppressed as usual. But the other half, carrying the duplication, showed vermilion eye color. That is, the duplication suppressed the suppressor. This shows that the duplication includes the locus of the suppressor and that the suppressor is a gene recessive to this wild-type allelomorph. If the suppressor had been a wild-type allelomorph of vermilion, then the presence of this duplication for the left end would have had no special effect upon the vermilion situation. The eye color would have remained red, *i.e.*, suppressed or "heterozygous" vermilion.

An even more striking demonstration was provided by the results of crossing these vermilion males, carrying vermilion and the suppressor in the X and also carrying the duplicating fragment, to females homozygous for vermilion and for the suppressor. The daughters (Table 2) of this cross were all homozygous for vermilion and for the suppressor, and half carried the duplication. The flies with the duplication were vermilion eyed, while those without it were the usual red (suppressed vermilion). In the vermilion-eyed daughters two doses of

the suppressor were themselves suppressed by one dose of the wild-type allelomorph carried in the duplication. The results of these two tests prove conclusively that the suppressor of vermilion is not a wild-type allelomorph of the vermilion gene but is a recessive mutant—a specific suppressor located at the left end of the X.

TABLE 2

SUPPRESSED VERMILION ♀ [su-v v/su-v v] × F₁ v ♂ [su-v v/Dup-101]

April, 1931	+ ♀ [su-v v/su-v v]	v ♀ [su-v v/su-v v/101]	+ ♂ [su-v v]	v ♂ [su-v v/101]
3,276	13	10	14	10
3,306	31	16	24	43
Total	44	26	38	53

Another of the early suppressors to which the duplication hypothesis was applied was that for sable. Since the locus of the sable suppressor is likewise at the extreme left end of the X, the same duplication, 101, should serve as a test here also. Females homozygous for sable and for the sable suppressor, phenotypically not-sable,

TABLE 3

SUPPRESSED SABLE ♀ [su-s s/su-s s] × Dup-101 ♂ [y sc/Dup-101]

March, 1931	Wild-type ♀	+ ♂ [su-s s]	s ♂ [su-s s/101]
3,216	15	9	6
18,878	12	4	3
3,191	115	30	21
3,193	51	13	13
3,194	64	16	20
18,875	22	13	7
18,876	91	34	39
18,877	89	34	28
Total	459	153	137

were crossed to males carrying Duplication-101. The sons (Table 3) which carried the duplication were sable, with spread wings; the sons carrying only sable and the suppressor were not-sable, and had the normal posture of wings. The spread wings are an accessory character-

istic of the sable mutant type. Thus, the presence of the wild-type allelomorph in the duplication dominated the suppressing action of the sable suppressor and allowed both the body color and the wing effects of sable to reappear.

Two separate stocks of suppressed sable were used in this test. The first was homozygous for the check character garnet (cultures 3,216 and 18,878), while the other was homozygous for the check characters tan, vermilion and garnet (remainder of Table 3). Both gave the same result with Duplication-101. It is thus clear that the sable suppressor is not a wild-type allelomorph of sable, i.e., a duplication, but is a recessive specific suppressor of sable.

Since the interesting black suppressor described by Plough ('27) is located also in the extreme left end of the X, Duplication-101 would serve for a test of the allelomorphism of this suppressor. Plough had already come to the plausible conclusion that since a single dose of the suppressor sufficed in the male, while two were necessary in the female, a gene mutation and not a duplication was involved. Our experiments with a stock supplied by Plough demonstrate this conclusively. Males carrying Duplication-101 and heterozygous for black were mated to females homozygous for black and for the suppressor. The mothers were homozygous for the recessive characters purple eye-color and curved wings, while the black chromosome of the father was marked by the dominant Plum eye-color (Muller, '30). Those of the Plum sons (Table 4) that carried the duplication were black, as expected on the assumption of a gene mutation (su-b/Dup-101; b/b Pm). The other Plum sons, without the duplication, had wild-type body color, suppressed black.

Some of the black Plum sons, in which the black suppressor was dominated by its wild-type allelomorph in the deletion, were crossed to stock females homozygous for the black suppressor and for black purple curved. The offspring (Table 5) were all homozygous for black

TABLE 4

Suppressed black ♀ [su-b/su-b; b/b] × Dup-101 ♂ [+/101; +/b Pm]

April, 1931	+ ♀	b Pm ♀	+ ♂	b Pm ♂	Pm ♂
	Mixed class	su-b/+ g b/b Pm	Mixed class	su-b/101 g b/b Pm	su-b g b/b Pm
3,310	72	49	38	19	23
3,314	55	38	33	15	12
3,316	29	27	22	14	16
Total	156	114	93	48	51

and for its suppressor, nevertheless that half which received Duplication-101 all showed black body color, since the wild-type allelomorph of the suppressor was introduced through duplication at the left end of the X.

In this case of black suppressor as well as the cases of vermilion and of sable suppressors, it is noteworthy that two doses of the suppressor are recessive to one wild-type allelomorph, thereby conforming to the general rule in triploid dominance.

These suppressors have been proved to be recessive mutant genes by the use of a duplication involving the locus of the suppressor. The technique involving use of a deficiency for the locus of the suppressed mutant could be applied to the purple suppressor found by Stern (*Z. f. Ind. Abst. u. Vererb.*) and by Bridges (in press, *Z. f. Ind. Abst. u. Vererb.*). These recessive suppressors, since they arose in a stock homozygous for the purple gene, might be interpreted as a small piece of chromosome II, containing the purple gene, attached to chromosome III. On this assumption four purple genes would be present in the suppressed purple flies, and the suppression would

TABLE 5

Suppressed black ♀ [su-b/su-b g b/b] × Dup-101 b Pm ♂ [su-b/101 g b/b Pm]

May, 1931	b Pm ♀ (101)	b Pm ♂ (101)	b ♀ (101)	b ♂ (101)	Pm ♀	Pm ♂	+ ♀	+ ♂
3,420	14	7	12	14	22	12	25	6
3,439	13	17	10	22	24	15	22	16
Total	27	24	22	36	46	27	47	22

be interpreted as the cumulative action on eye color of genes which in two or three doses could only make a purple color. The large secondary effects in the case of Stern's suppressor, including high inviability, complete sterility of both sexes, and strong reduction of crossing-over, served to make the duplication hypothesis still more plausible.

A viable deficiency for the purple locus was provided by the deficiency from Translocation-H (Morgan, Bridges, Schultz, '30), in which a small section taken from near the center of the second chromosome, and including the loci for hooked and purple, is attached to the Y chromosome. The H-deficiency can be detected by the small bristles and other structural peculiarities of the flies heterozygous for it. Thus it was a simple task to obtain flies homozygous for the purple suppressor carried by chromosome III, and carrying purple in one chromosome II, while the other chromosome II is deficient for the purple locus. If the suppressor is a duplication carrying the purple gene, in such individuals the number of purple genes is reduced by the deficiency to three, and hence the eye color should be again purple. The situation would be analogous to that in the purple-eyed fly homozygous for purple but heterozygous for the suppressor. If, on the other hand, the suppressor is not a purple gene but is a true third-chromosome gene having a recessive specific suppression effect in purple, then the deficiency for purple should not change the situation with respect to the suppression and the fly should remain wild-type, *i.e.*, suppressed purple.

In testing this hypothesis males were synthesized which carried black purple in one chromosome II. The other chromosome-II was Deficiency-H, while Duplication-H was carried by the Y-chromosome. One third chromosome carried the recessive purple-suppressor (Stern's) and the other the dominant marker Hairless. Such a male was mated to a female homozygous for black purple and likewise having Stern's purple suppressor in one chromosome III and Hairless in the other. In the off-

TABLE 6

(+/+, b pr/b pr, su (S)/H) ♀ × (+/Def-H, b pr/Def-H, su (S)/H) ♂

Phenotype	b pr H ♀	b ♀	pr Def H ♀	Def ♀	H ♀	b H ♂	H ♂	b ♂	+ ♂
Genotype	+/+ b pr/b pr su/H	+/+ b pr/b pr su/su	+/+ b pr/Def su/H	+/÷ b pr/Def su/su	+/+ Dup b pr/Def su/su	+/Dup b pr/b pr su/H	+/Dup b pr/Def su/H	+/Dup b pr/b pr su/su	+/Dup b pr/Def su/su
Feb. 1931									
3,076	31	7	4	1	1	29	23	2	16
3,108	45	17	11	1		34	62	23	29
3,210	20	3		1		16	15	1	8
3,215	44	3			1	24	30	2	6
3,217	24	3	1			13	16	2	10
Total	164	33	16	3	2	116	146	30	69

spring the deficiency appears in the daughters only. The daughters (Table 6) carrying Hairless were only heterozygous for the suppressor, but the not-Hairless daughters were homozygous for the suppressor. The daughters carrying the deficiency, easily recognized by their small bristles, etc., and homozygous for the suppressor, distinguished by being not-Hairless, in the only three individuals found, were wild-type in eye-color and not purple. Hence the suppressing action was not affected by the deficiency, and the suppression is accordingly not due to an additional purple gene but to a mutation of a third-chromosome gene. This is further evidenced by the lowered viability of the combination of Deficiency-H with the suppressor, which, were it a duplication for the region of purple, might be expected to neutralize the effects of the deficiency. Since neither in the somatic effects of Deficiency-H nor in its viability is there any neutralization, but instead a super-position of the phenotypic effects of the suppressor and the deficiency upon each other, on this count also, the suppressor must be considered as a mutant gene.

The test was extended to the purple suppressor found by Bridges (in press), which suppressor, while allelomorphic to Stern's suppressor, showed certain differences, including higher fertility. The compound between them has still higher viability and fertility, higher than either showed in homozygous forms. Females homozygous for purple and carrying suppressor-B in one third-chromosome with a dominant marker, Stubble, in the other, were crossed to the same type of male used above. The not-Stubble, not-Hairless offspring were suppressor (S)/suppressor (B) compounds. The two which carried deficiency-H (females only) were found to be wild-type in eye-color and not purple-eyed (Culture 3,298). Thus, the purple suppressor found by Bridges is likewise not a purple duplication but is a mutation of a gene normally present in the third chromosome.

The same type of experiment, differing principally in that the suppressor entered through the reciprocal parents, furnished the data of Table 7. Here again the

deficiency for purple did not undo the suppressing action of the third-chromosome mutant genes, as shown by the red-eye color of the seven Deficiency-bearing females.

The experiments reported in this paper have completed the proof that the suppressions in the cases discussed are due, not to section duplications, but to monogenic mutations in loci other than that of the gene suppressed. The several types of proof—through use of a deficiency, a duplication or a polyploid condition—are generally applicable, and, on the whole, of equal theoretical validity. As is obvious, certain loci can best be treated with a particular method, so that no extended discussion of the comparative merits of the different methods is called for. The triploids, 3N, generally present the greatest technical diffi-

TABLE 7

$(+/+,\ b\,pr/b\,pr,\ su(S)/II)\ \female \times (+/Dup\text{-}II,\ pr/Def\text{-}II,\ su(B)/II)\ \male$

Phenotype		pr H ♀	+ ♀	pr Def H ♀	Def ♀	H ♂	+ ♂
Jan. 1931 Genotype	I	+/+	+/+	+/+	+/+	+/Dup	+/Dup
	II	b pr/pr	b pr/pr	b pr/Def	b pr/Def	b pr/pr or Def	b pr/pr or Def
	III	su/H	su(S)/su(B)	su/H	su(S)/su(B)	su/II	su(S)/su(B)
3,039		41	18	15	7	68	42

culty, but on the other hand are not limited to those cases in which a duplication or deficiency has chanced to fall into our hands. By use of the x-ray method an increasing number of deficiencies and duplications are becoming available.

The bearing of these results upon Fisher's theory of evolution of dominance (Fisher, '30—a review) is obvious. The suppressors reported here are demonstrated to be mutant genes similar to those which Fisher has postulated to maintain the dominance of the normal allelomorph.

They differ in that they affect the phenotypic appearance of the homozygous recessives rather than the heterozygotes important in the consideration of dominance. But suppressors of dominant mutants in the heterozygous

condition are known, *e.g.*, the third-chromosome recessive suppressor of Hairy-wing. Yet, generally speaking, they are less fertile and viable than their sibs which do not carry the suppressors. Selection would, therefore, operate against most of them, as it does against the large majority of all new mutations, which indeed Wright ('29) has pointed out. There are, however (Bridges, '32, in press), some cases—*e.g.*, port suppressor—in which the suppressed form has been known to replace the original mutant type in a stock containing both. Such cases have been considered as supporting the theory offered by Fisher (1930, p. 55).

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RADIUM AND LETHAL MUTATIONS IN DRO–
SOPHILA

Further Evidence of the Proportionality Rule from a Study of the Effects of Equivalent Doses Differently Applied[1]

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THE fact that irradiation of some species of living things induces gene mutations led to the suggestion that variability among organisms in nature might be due, in part, at least, to radiation from naturally-occurring radioactive substances, the implication being that such radiation might have played an important rôle in the evolution of species. Results subsequently published by several investigators have seemed to indicate that such might be the case.

Olson and Lewis ('28) were among the first to point out the desirability of testing experimentally the effects of natural radiation upon organisms. Babcock and Collins and the present writers, working independently, performed almost identical experiments to test this point. Using an electroscope, Babcock and Collins ('29) discovered a location in a street-car tunnel in San Francisco where the natural ionizing radiation was fully twice as great as the radiation in their laboratory in Berkeley. Accordingly their experiment was designed to compare the rates of occurrence of sex-linked mutations in Drosophila in the street-car tunnel and in the laboratory. Of 3,481 tests made in Berkeley 9 hatched no male flies, or a rate of 0.26 per cent., of lethal mutation. Two thousand five hundred tests made in the tunnel gave 13, or 0.52 per cent., of lethal mutation. While the difference

in rate, 2.5 times the probable error, is not fully significant statistically, these authors considered that the results approached significance.   Upon a reanalysis of the data showing the actual experimental variation in rate in the several subgroups in each of the two series, it appeared that the difference between the average rates for the two locations was increased: 0.275 ± 0.086.

The present writers ('30) found that in an abandoned carnotite mine in the East Paradox Valley of Western Colorado the air was strongly ionized.   In addition to the electroscope readings an attempt was made to compare the amount of natural ionization in this mine with that of one milligram of radium.   Radiation in the mine was 0.39 times as intense as that from one milligram of radium when the rays were passed through a 0.156-inch lead filter.   There were 2,860 test cultures, of which 7, or 0.245 per cent. ± 0.062, produced no male flies.   In the 1,308 control cultures one lethal mutation occurred, a percentage of 0.076 ± 0.051.   The difference between tests and controls is 0.169 ± 0.081, a difference 2.09 times its probable error.   The authors believed that, while this difference was theoretically not statistically significant, it might actually be so, and that had the flies been exposed for a much longer period, the results would have been more striking.   In this connection it was suggested[2] that a series of equivalent amounts of radiation might be carried out in the laboratory and the time of exposure extended accordingly.

These two experiments, one in California and one in Colorado, although falling short of statistical significance, were consistent, nevertheless, in that both gave an actually higher rate of mutation in flies exposed to natural radiation than in the controls.   Thus it seemed that these results strengthened definitely the plausibility of the suggestions that natural radiation might be responsible for the mutations which ''are the grist of the natural selection mill with the resulting evolution of new forms.''[2]

2 Hanson and Heys, 1930.

Shortly after the publication of these results, however, Muller and Mott-Smith ('30) reported measurements which indicated that natural radioactivity is far "inadequate to explain the frequency of natural mutations." Calculations were made of the relation between the artificial and natural radiation effects in order to compare the induced and natural mutation frequencies. The measure of radiation intensity in both cases was the usual ionization per cc per second in air. Considering radiation from every possible source (outer environment, culture medium, the flies themselves, etc.), and taking the lowest average natural mutation value from observations by Muller, Altenberg, Babcock and Collins, Hanson and Heys, and others, Muller and Mott-Smith arrived at the following comparison:

(a) natural mutation—without special treatment—1:150 of the highest value artificially induced;

(b) ionization values—7.2×10^{12} ions per cc for artificial treatment and 3.6×10^{7} ions per cc for untreated material, or a ratio of 1:200,000.

These authors consider that the difference between the ratios, 1:150 and 1:200,000, is undoubtedly significant, particularly since for comparison they chose "the minimum possible value for the natural mutation frequency and the maximum value for the artificially induced mutation frequency, the maximum for the natural ionization and the minimum for the artificially induced ionization."[3] This makes the natural mutation frequency "at least thirteen hundred times as high as it would be if it were caused solely by the radiation which the flies receive from their 'outer environment,'" and leads to the conclusion "that natural radioactivity is not the major cause of mutations, and of organic evolution, but that most mutations come about as a result of other causes."[4]

The validity of the foregoing conclusions was admitted by some only with great reluctance, and in this connection it was suggested that perhaps the small amount of

[3] Muller and Mott-Smith, 1930, p. 279.

[4] *Ibid.*, p. 283.

natural radiation had a disproportionately great effect owing to its being spread over so long a period of time.

Experiments by Hanson and his associates ('28, '29ᵃ, '29ᵇ, '31) both with radium and x-rays have shown that the induced mutation rate obtained always varied directly with the dosage as measured by the ionization in air, indicating a striking proportionality between the physical agent and the consequent biological effect. Oliver ('30), using five x-ray dosages, varying only in time factor, found the total number of lethals occurring directly proportional to the duration of the treatment. Demerec and Farrow ('30) have reported results indicating that at low dosages the increase in the percentage of primary non-disjunction is "almost proportional" to the x-ray dosage applied.

In spite of these findings, however, it seemed possible that the proportionality rule might hold over only a limited range of duration or dosage and not perhaps for durations of days or weeks at very low dosages.

Procedure

Accordingly experiments were initiated with a view to determining within what limits the proportionality rule holds. A series of dosages as nearly exactly equivalent as possible were planned which were to be applied in different ways, that is, in one case high intensity for a brief period as compared with an equivalent dose of low intensity spread over a long period of time. The method of irradiation was such, it is believed, that gamma-radiation only reached the flies and all secondary beta-radiation was avoided. The alpha and beta-rays were excluded by means of a 0.5 mm platinum filter, which at the same time allows the passage of seventy-eight per cent. of the gamma-radiation. The possibility of secondary beta-radiation was eliminated further by filtration through a layer of specially prepared wax one centimeter in thickness, and by enclosing the flies for treatment in gelatin capsules. This supplementary filtration through wax

also serves to diffuse the rays giving even irradiation.[5] In order to provide a food culture medium during the long treatments and yet avoid secondary radiation from glass containers, heavily paraffined paper boxes were employed as treatment chambers in place of gelatin capsules. Just how great a secondary radiation is set up by gamma-ray vibrations upon entering the body of the fly is a matter of speculation. Wild type males were used, as in previous experiments, and after exposure were mated to heterozygous bar-eyed females of the C1B stock.

An attempt was made to secure exactly equivalent dosages in two series as follows:

    I. 300 mgms radium, ½ hour's exposure—6315.00 r-units.
       4 mgms radium, 37½ hours' exposure—6315.75 r-units.
       2 mgms radium, 75 hours' exposure—6315.30 r-units.
   II. 300 mgms radium, 1 hour's exposure—12,630.00 r-units.
       4 mgms radium, 75 hours' exposure—12,631.50 r-units.
       2 mgms radium, 150 hours' exposure—12,627.00 r-units.

As a check on these, a heavy treatment of 4 milligrams for 150 hours (25,263.00r) was given. The distance in each treatment was the same, 10 centimeters. In order to be certain of equivalent doses in each case, ionization readings for the amounts of radium used were taken by means of an electroscope, and the total dosage translated into r-units according to the usual method: the reciprocal of the fall of the needle in seconds multiplied by the constant, K, gives r-units per minute. Since gamma-rays ionize a gas very slightly, radiation which is composed solely of gamma-rays can not be measured except by the secondary radiation it produces. Thus the calibrations given are for the amounts of radium used with filtration through 0.5 mm of platinum.

EFFECTS OF EQUIVALENT DOSAGES COMPARED

Upon scanning the sterility data given in Table 1, it is apparent that the percentages of sterility in both the

[5] Watson and Lawrie, 1931.

parent and the F_1 generations are approximately directly proportional to the dosage, regardless of the mode of application.   In the two series of equivalent dosages there is no difference among the resulting sterility values which approaches significance, the differences being several times less than their respective probable errors. These sterility values are absolute in that they represent complete sterility.   In all matings where sterility was suspected a period of recovery was allowed, and any return to fertility noted.   Males which showed apparently permanent sterility were remated to new females as a test. Complete permanent sterility is a new observation in this work.   It is interesting to note that sterility in the F_1 generation follows the dosage closely, and that the F_1 sterility values in each instance are approximately one half those for the treated generation.   Since the F_1 individuals are one generation removed from exposure, this result suggests perhaps the existence of some factor necessary for fertility which was affected by the treatment.

TABLE I

STERILITY DATA

Generation	Dosage in mgms Ra	Exposure time in hours	Total number of cultures	Number of sterile cultures	Percentage of sterility
$P_1 \times P_1$	300	$\frac{1}{2}$	187	8	4.2781 ± 0.9960
	4	37$\frac{1}{2}$	150	7	4.6666 ± 1.1616
	2	75	150	6	4.0000 ± 1.6000
	300	1	178	16	8.9887 ± 1.4451
	4	75	150	14	9.3333 ± 1.6018
	2	150	150	13	8.6666 ± 1.5499
Heavy dosage	4	150	150	25	16.6666 ± 2.0528
$F_1 \times F_1$	300	$\frac{1}{2}$	650	13	2.0000 ± 0.3704
	4	37$\frac{1}{2}$	650	14	2.1538 ± 0.3841
	2	75	650	15	2.3076 ± 0.3892
	300	1	650	24	3.6923 ± 0.4988
	4	75	650	28	4.3077 ± 0.5371
	2	150	650	31	4.7692 ± 0.5637
Heavy dosage	4	150	400	34	8.500 ± 0.9394

TABLE II

Showing the Lethal Mutation Rates and the Dosages

Dosage in mgms Ra	Exposure time in hours	Ionization proportional to	Dosage in total r-units	Number fertile F_2 cultures	Number lethal mutations	Percentage of lethal mutations
300	½	0.2105	6315.00	637	30	4.7095 ± 0.5661
4	37½	0.002807	6315.75	636	30	4.7169 ± 0.5670
2	75	0.001403	6315.30	626	29	4.5669 ± 0.5616
300	1	0.2105	12,630.00	626	61	9.7444 ± 0.7980
4	75	0.002807	12,631.50	622	60	9.6463 ± 0.7543
2	150	0.001403	12,627.00	619	59	9.5315 ± 0.7938
4	150	0.002807	25,263.00	366	74	20.2186 ± 0.4474

Table II shows the lethal mutation rates and the dosages in intensity and time and in r-units. The differences between the largest and the smallest mutation figures obtained in each series fall short of significance:

4.7169 ± 0.5670	9.7444 ± 0.7980
4.5669 ± 0.5616	9.5315 ± 0.7938
Difference 0.1500 ± 0.7980	Difference 0.2129 ± 1.1256

The heavy test treatment gave correspondingly higher sterility and lethal mutation rates. Fig. 1 shows these results graphically.

DISCUSSION

The above observations seem to indicate that even within these fairly wide limits the proportionality rule still holds, and the frequency of mutation produced corresponds with regularity to the energy of the dosage absorbed. Equivalent doses give as nearly approximately equivalent results as might well be expected in dealing with living things. The long continued release of electrons by a radiation source of low intensity produces sterility and mutation results equivalent to, but not greater than, a corresponding dose of high intensity and short duration.

A1-300mgms. 1/2 hour-6315.00r
A2-4mgms. 371/2 hours-6315.75r
A3-2mgms. 75 hours-6315.30r

B1-300mgms. 1 hour-12,630.00r
B2-4mgms. 75 hours-12,631.50r
B3-2mgms. 150 hours-12,627.00r

C-4mgms. 150 hours-25,263.00r
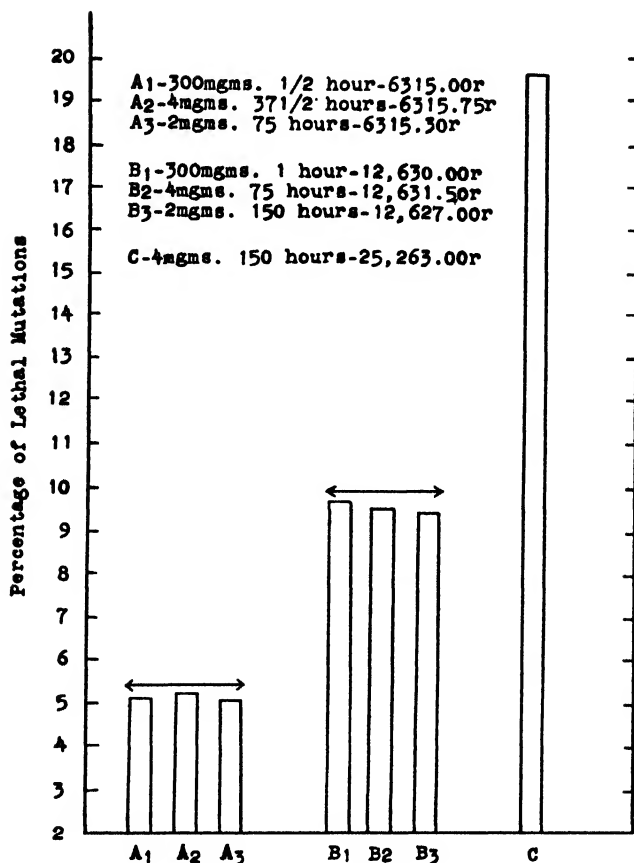
Fig. 1

Accordingly a small amount of radiation, such as that from naturally-occurring radioactive substances, does not seem to have a disproportionately great effect owing to its being spread over a long period of time. If, as the measurements and calculations of Muller and Mott-Smith show, the total radiation of the ordinary environment taken at its maximum is not adequate to explain the mutation frequency observed in the absence of treatment, and if, as the present observations indicate, dependence for induced mutation is strictly upon the total energy absorbed, regardless of the exposure period, we are still at a loss to account for the majority of natural mutations, and the question of the major cause of variation in organisms remains unanswered.

One can picture conceivably the occurrence in living tissues of a slow spontaneous process which might be accelerated slightly by natural and to a larger extent by artificial radiation. Studies of the differences in the susceptibility of cells under varying physiological conditions give evidence that the physico-chemical state of the cell at the time of irradiation influences radiation effectiveness. The authors are conducting at present experiments along this line.

When gamma-rays, which are of high vibration frequency and the quanta of which are, therefore, great in energy content, strike the atoms of cell substances, the transformed energy sets up both physical and chemical changes. The atom absorbs energy, has· momentarily a greater "chemical sensitivity" or reactive ability, and can enter into combinations previously impossible. Whether chemical combinations already in progress are accelerated in the same direction or disturbed and sent off in another direction by this transformed energy is a matter of conjecture. However, Muller ('30) has pointed out from his own work on temperature and from a careful analysis of the results of Oliver with x-rays ('30) that applied radiation does not seem to act as a catalyst. It is conceivable also that the threshold of disproportionality has not been reached within even the very wide limits arbitrarily set in these experiments.

Summary

Experiments planned with a view to determining within what limits the proportionality rule holds show again a strict correspondence existing between the amount of radium administered and the consequent biological effect, the induced mutation frequency obtained varying directly with the dosage.

A series of dosages as nearly exactly equivalent as possible but applied in different ways gave approximately equivalent results, dependence being merely upon the total energy absorbed. Whether the treatment con-

sists of a high intensity and a short exposure or low intensity and long exposure the dosage expressed in r-units is remarkably constant.

Within the limits set for the present investigation (2 mgms radium for 150 hours to 300 mgms radium for 1 hour) a small amount of radiation does not seem to have a disproportionately great effect owing to its being applied over a long period of time. Perhaps disproportionality begins outside even these wide limits, and, if so, there is still a possibility that a small amount of natural radiation acting over a very long time might be sufficiently effective to account for natural mutations.

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THE STATUS OF THE SPECIES AND THE GENUS

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I. STRUCTURES AND ORIGIN

THERE seems to be no valid species, based on a single character. So-called physiological "species," differentiated by a single physiological character, are typical varieties, not subspecies, which are geographical units (see below). Any one species in a genus is differentiated from every other species by a *set* of morphological, as well also as physiological and habitudinal characters. Therefore a species originates, not by a change of one character, but by several contemporaneous changes.

Such combination changes are claimed to originate in three ways:

1. *By Hybridization.* This may occur where species overlap or where an individual is suddenly dropped into a new locality. If this field is fruitful, novelties should be sought for chiefly where congeneric species overlap.

2. *By Environmental Stimulus.* That there is such a thing has been amply proved by the North American Fauna series of the U. S. Biological Survey where one finds that desert regions consistently imprint certain characters on its mammals (also true of birds, snakes, insects and others), that humid areas consistently imprint their characters, dune areas theirs, etc., so that definite subspecies are found in each of these habitats with certain characters modified in the same way. These subspecific (geographical) characters have since been found to be constant on transplantation. Further, these changes check with "industrial melanism" in Lepidoptera (1), which phenomenon ought to be checked in the Pittsburgh region. Finally alpine dwarfing retains its characteristic in sea-level gardens.

Such environmental changes are known as mass changes, because they affect all the individuals of a popu-

lation subequally. I fail to find, however, a record of such subspecific (geographical race) variation being intense or extreme enough to be considered a full species. If, for instance, the geographical color change continued, the animal would eventually become albinistic—and albinism is not by itself a specific character, if it ever is. The same is true of melanism or any definite color. In other words *all* the colors of the species are equally affected. The pattern remains essentially the same, no new colors are added. Similarly, changes in size as dwarfing or its opposite are not the specific changes of changes in proportions. There are no combinations of structural changes in these subspecies. Thus geographical races are easily referable to their specific type. Such subspecies are, therefore, a standard systematic unit and throw no light on the origin of *species*.

(The same may be said of mutational varieties like albinism, xanthochroism, erythrochroism, etc., involving the loss of some *one* character or its appearance.)

The ornithologists—who have worked up their group more intensively than is the case with any other group, due in large measure to its huge corps of workers and its very early interest—have recognized these subspecies to the extent of ear-marking them as groups which freely interbreed. Objection has been raised to their stand (2, p. 54), on the basis that some western subspecies do not show interbreeding. Where this is true, it is for the very evident reason that whereas in the east, subspecies are limited by very gradual transition areas, in the west they are often separated by high mountain ridges, desert areas (affecting Cynipids), and other barriers so sharply segregating the subspecies as to make it impossible for them to interbreed. The ornithologist's ruling was merely intended to safeguard the species rank and was not intended as a definition of species and subspecies—hence is not comprehensive (inclusive of all subspecies). If the clause: ''where interbreeding is geographically possible,'' were inserted, it would tend to forestall misunder-

standing. The fact remains that the ornithologists (as also the mammalogists) recognize the subspecies as definite geographical races of their species, freely interbreeding where the ranges overlap or meet (that is, on transitional belts). This is tantamount to saying that geography makes subspecies, but not species. A conchologist, who has spent many years in intensive study of fresh-water Mollusca of the United States, has found it necessary to adopt the same principle: "to consider a group of individuals which are separated from all other groups by some definite *combination* of characteristics, without intergrading, as species; those that show intergradations as varieties (3, ¶3).

3. *Mutations.* Our knowledge on this phenomenon is still so slight as to make it undesirable to attempt inferences. *Combinations* of specific characters, as is well known even to geneticists, are locked and can not yet be broken up by man. In Drosophila, for instance, by intensive inbreeding many aberrations have been produced, but none of the old specific character *combinations* (species) have been produced, no new ones made. The statement, "Since most of the mutants are *weaker* or less well adapted types than the wild type, they disappear before they are recognized" (4, p. 65), is undoubtedly intended more especially for bottle Drosophila. What do we know about mutations in the wild? What do we know about the contemporaneous mutation or recombination of *specific* (not aberrant) characters? Each genus, as it were, is a combination lock, and each of its species is a specific combination which is changed after a lapse of time. The species characters are impressed on each of several discs (chromosomes) and man has not yet learned to make up combinations (barring aberrations).

We might note in passing the effect of gregariousness and sedentariness as contrasted to the opposite habits on the rapidity of evolution. According to present concepts a recessive factor (incipient character) originating in an individual may be transmitted by breeding to many in-

dividuals, as a unit factor (possibility), but that it will not become evident (materialize) until two individuals bearing this recessive gene and of opposite sexes cross (4, pp. 59–65; 5, pp. 248–249). Thus a recessive factor which pops up in an individual of a species of animal having a tendency to live secluded in a clump of moss, or in a grass tussock or mat, will have far more of a chance of being disseminated throughout its companions and thus of becoming an actual character (by the union of that pair of recessive genes) than if it had occurred in a species having a tendency to climb trees every evening and then parachute off every morning. Likewise, such recessive characters would materialize much more quickly in a species of Mollusca hemmed in by high mountain walls or confined in an isolated group of trees than in a species ranging widely or promiscuously over a grassy plain. The tendency to live in herds or of returning after every migration to the same nesting site would also be advantageous to such rapid materialization of recessive factors. As a final example, a species living on mountain sides and having a tendency to ascend would become more and more concentrated at the top of the mountain because of its converging slopes. Thus mountain tops become centers for more rapid evolution. In brief, since inbreeding tends to bring out recessive mutations (not to mention dominant), evolution should be most rapid wherever the individuals of a species become concentrated, or lack the tendency to disperse.

II. Structures and Habits

In all three of these reputed originators students have been more concerned, like the systematist, with structure. "The Species Problem" (6) presents instance after example to show that there are exceptionally few real cases of correlation of structure with adaptation or selection and that in such few cases habit needs be also a correlative.

Habitat or any habit may be as much a specific character as the structural character, originating as an independent unit character, accompanying the morphological characters and subject to all the laws of specific unit characters in transmission and heredity (6; 5, p. 315 last word ff.). Because we can easily see, measure and describe the structural characters, we use them for species differentiation; the habits are lost in alcohol. It is the habits, however, that determine the numbers of a species in its food relations, protective relations, propagative relations. There is no better example of this than the study of the two British hares (6, pp. 197–202) in which the attempt is made to determine by a careful analysis of the specific differences in structure "how . . . structural differences . . . may be *explained by* the habits" for the supplanting of the one species by the other. The conclusions (p. 202) are that, "in Great Britain at least, the structural differences do not, with two possible exceptions [one *very* dubious], *correspond to* two radical differences in habit and mode of life in relation to which they have been of selective value." Following on, one finds the cue to the differentiation of habitat: "The Brown Hare has supplanted the Blue Hare very possibly on account of the boldness and more confiding temperament [more inquisitive, on p. 198] of the latter which, as the country was brought into cultivation and more systematically hunted over, laid it open to destruction by men and dogs." This is a familiar story concerning several of our American wild animals. "Other examples (p. 209) are to be seen in the success of the Brown Rat (*E. norvegicus*) at the expense of the Black Rat . . . , of *Lepus americanus* at the expense of *L. arcticus* in Newfoundland" and others, or of *unsuccessful* attempts at introducing certain species.

After a careful review of all that has been done in the past eighty years to prove the value of interspecific structural adaptations in natural selection, the conclusion is reached (6, pp. 217–219) that we have no certain evi-

dence. If it did exist, after so much observation and experimentation, should it not be plainly evident? (Even the value of mimicry has been strongly questioned (7, p. 432). Finally all things failing, we are asked (6, p. 185, ¶1) not to be too rigid; not to insist on absolute correlation!

Again, a protistologist (8, p. 24, last ¶) found that in the dinophysoid flagellates, which are pelagic, there is no evidence of isolation and that ''very closely related forms are not infrequently recorded in the same surface catch.'' By surface catch is meant uppermost fathom. ''The ecologic niche which each [species] fills is in reality a wide shelf girdling the tropical seas and extending to a considerable depth. Moreover, many of these areas of distribution appear to be to a large degree coincident, even within the species'' most closely related. This is undoubtedly true of most truly pelagic microscopic forms of life. Such results give us reason to doubt the efficacy of specific structures, or even habitats in determining survival.

Certainly it is not the structural, taxonomic ear-marks of the species that determine an animal's (or plant's) degree of adaptation to its environment or give it supremacy. but its habits, more probably breeding habits. Natural (congeneric) selection is operative far more through habits (and habitat preference) than through superficial structure, and it is not until the morphological differences become so marked as to be of generic or family value (as reiterated by Robson) that they become of adaptational (selective) value and thus supersede the value of habits in this respect. Let us henceforth turn our eyes more and more, then, on habit studies as of *early* evolutionary significance, even though we must change from systematist to ecologist (sociologist).

Habitat restriction, by and large, becomes of no adaptive significance in the sense of a function of structural characters, but may be a specific character like color of nape, or spine of shoulder, or a habit acquired through

competition or restriction of range.   Habitat preference
in plants is in the nature of an adjustment (physiologi-
cal) rather than an adaptation (structural).   Were
environment of such significance or so potent a factor, all
species of an old Paleozoic or Mesozoic (unglaciated)
area like some parts of China, would have the leaves of
plants like Aster, Taraxacum, Erodium, Chenopodium,
Viola, Astragalus, etc., growing side by side, all say strap
shaped and hirsute; and the animals also would be re-
duced to the same superficial appearance through envi-
ronmental convergence.   The fact is, however, that other
factors operate to keep species of unrelated genera and
higher groups *different,* while the leaves, etc., of con-
generic species are more similar than are those of unre-
lated species.   It is only when the environment is so
extreme (as in deserts, alpine regions, etc.) that it can
counteract other influences enough to *visibly* impress
itself and bring about structural convergence to any
extent.   In general, therefore, the physical environment
is impotent in speciation, and any cases of special fitness
of species in a not extreme environment would be excep-
tional rather than the rule.   This seems also, by its nega-
tive results, to be the vintage of the years of research.
Structural characters of specific rank are facilities to the
systematist but only rarely of immediate value to the
species in its livability.   Our point of view has been
extremely anthropocentric.   ''The Species Problem'' is
a monument to the lack of adaptational value of specific
structural characters, and coming from an English biolo-
gist is of double moment.

III. Generic Development

On the above basis, a species may be described as life
forms related to each other, by the possession of the same
combination of structural characters which are usually
so poorly developed as to be of no immediate adaptive
or selective value in the process of evolution, as well as
by the possession of a combination of similar physiologi-

cal and habitudinal characters. The structural characters are so small or poorly developed that the environment has no effect on them. These various specific characters continue being modified (from within) and recombined (within the genus) to form, through time, more and more species until the generic area becomes so crowded as to bring about congeneric relations. Thus congeneric competition develops through such non-morphologic characters as "temperament": aggressiveness, recessiveness, inquisitiveness, audacity, pugnacity, secretiveness; vitality statistics (birth rate, fecundity, number of broods, care of young, etc.) and other sociological relations and habits. As examples of the intrageneric fitness (not necessarily competition) see 6, p. 209. Thus by intrageneric (congeneric) fitness or superfitness (not structural adaptations) the "weaker" species are crowded out, or more and more restricted in their distribution or niche while the more successfully prolific, the more viable, extend their area until the genus has many species throughout the zone or globe, some restricted, some more extended and one holotropic, holarctic or circumpolar. From this time on, generic senility occurs—due largely to the competition between the various congeneric species and the wide. Finally one of two things may occur: (1) the wide may become the single representative of the genus—as in Nyctea (snowy owl), Scotiaptex (great gray owl), Surnia (hawk owl), Cochlicopa (Mollusca) and many invertebrates, or it may occur with a few species (as often), (2) the wide itself may drop out leaving a few widely separated (relict) species—as in Bombina (Amphibia).

To summarize: A sedentary or gregarious "parental" form (phylogenic genotype) throws off various combinations of characters, each at a different center, each of which, by the growth of reproduction, advances from its center like an eddy across the country, influenced in its spread chiefly by "barriers." Each offshoot begins from the parent at a different time and place and is held

constant by heredity. The eddies of range extension may be in any direction or have a general trend, depending on local factors governing distribution of species. At any time, however, some of these radiating species may in the course of migration meet a confrère with which it is capable of interbreeding and thus in turn become the center of origin of a radically new type as a subgenus or genus. Such may be illustrated by Artemisia (9, pp. 34, 49), Cynips (Hymenoptera, 2), Thamnophis (Ophidia, 10) and others. If there is any truth in emergent evolution, as there undoubtedly is, these crossbreeds would produce something new and would not necessarily show combinations of the old elements (parent stocks). If this interpretation be correct, phylogenists should seek the parental species among the most sedentary or gregarious of the group.

As emphasized in "The Species Problem," it is only when structural differences are of generic, more especially of family rank that they are positive enough to be of *selective* value. This should be paramount to saying that families are of greater evolutionary significance than species and therefore more rapid in their development and senescence. Taxonomically, the genus is the problem.

IV. The Question of Survival

Darwin, in order to bolster up the survival of the fittest end of his theory, very much overemphasized mortality statistics. More careful studies of living forms have shown that the eliminated are to a large extent the young, the old and the diseased. In the case of some species (salmon) the death rate among the old reaches nearly as high as 100 per cent. per annum. Provided that they are not eliminated before spawning, their elimination is of no consequence to the species. In other cases, say birds, it is usually the older that are eliminated, or, according to Howard (11), the males when too numerous.

According to recent work in ecology, a climax forma-
tion, as a beech or spruce woodland, is a balanced as well
as a closed association. In such an environment, a new
species would have more chance for survival from preda-
tors than the old because the new would be correspond-
ingly more rare (say one or more couples among tens of
thousands of the old). And it is not until its numbers
would be equal to those of the old species that it would
become equally eliminated (other things being equal—
and structural congeneric characters almost always are).

For example: Suppose the new species be a brown-
winged warbler with one white cross-bar, a black line
over the eye and a white bib, otherwise like the blue-
winged warbler of the northeastern United States. Such
a combination of color is no more conspicuous than any
of the other warblers. Its size may be a few millimeters
(a quarter of an inch) longer or shorter than the blue-
winged warbler, the relative length of tail, wings, bill,
may also be a few millimeters different. All these dif-
ferences are of no particular advantage. Its numbers
are two, a male and a female (one could begin with less).
Would its chances of survival be less than those of any
other warbler? If its disposition were such as to perch
on the end of exposed twigs to sleep, it would undoubt-
edly be eliminated in short order. If its psychic habits
were about the same as other warblers, it would not be
wiped out any earlier than the neighboring pair of a
related species. If it is associated with nine pairs of
blue-winged warblers in a wood, it would stand the
chances of one in ten of being killed by warbler enemies
(high mortality being during youth, including eggs, or
old age). If it were more secretive in disposition its
chances of survival would be greater, if less secretive
less. There would be no elimination by mating rivals
because there would be no male competition, as males of
another species would not recognize it as a rival. If its
egg complement and care of young were similar to that
of the blue-winged warbler it would increase normally

until as numerous as the blue-winged warbler, provided that the woodland's capacity for it were also nine pairs. The number would then remain at nine, neighboring woodlands receiving the surplus (or squirrels, snakes, etc.). If the egg complement were greater, it would increase to woodland capacity more rapidly. If each male were satisfied with a smaller territory than the blue-winged warbler that woodland would contain a greater number of pairs at optimum increase. The question of available food is one which varies with insect population, from year to year, and ability to vary its food habits. Degree of security from enemies varies with density. For instance a snake or squirrel would have to scrutinize fewer bushes or trees to find a nest of young if there are thirty nests in a wood than if there are nine. Also the more frequently the snake feeds on one kind of animal, the greater are its chances of getting heavily parasitized and thus weakened. This ratio between density of prey and degree of parasitization may account for the relatively low numbers of predators. As soon as prey fall below a certain number, the normal predator meets with other food while seeking for the few of its normal diet. Thus a balance is kept, not so much by habits as by ratio of size of prey relative to size of its habitat or cover, relative to the covering ability of its predator. Numbers in excess of this optimo-minimum is of no advantage to itself unless the prey harbors a parasite of the predator.

Exactly the same conditions would obtain for herbivores except that their food supply is less precarious. Furthermore, the smaller the life form, the less chance for elimination because of additional ease of secretiveness and the much greater distances that must be covered by the predator.

Thus it may be seen that the real question of survival of new species is not dependent upon small morphological characteristics (always with rare exceptions) but first, one of propinquity (both temporal and spacial) of one

male and one female and second, temperament, disposition, habits, especially breeding habits, and such characteristics.

As to food "competition" with the old species, fluctuations in numbers (12, 13), which is probably less frequent in climax associations, would bring in a chance factor to the extent that when the species is numerous in individuals, it is more quickly consumed, a rare remnant almost always being left. Near exhaustion of food supply often leads to migration of the predator. Different conditions would occur in different groups of plants and animals. Generalities of this sort are discussions in vacuo. The field of sociology is to secure the data for each species under its various conditions. My experience in unbalanced (non-climax) woodland is that there is a great deal of "room" to spare, either for sunlight or for absorption; either for nests or for food. As a single factor, consider the number of leaves that fall in the autumn, uneaten. In north China, the pagoda tree (*Sophora japonica*) is entirely defoliated in June by the Geometrid moth *Macaria cinerearia* (14) and immediately puts out a second crop of leaves. This is also the case with *Ulmus pumila* in late August, new leaves coming on even so late in the season. But out of fifty neighboring trees of *S. japonica* on the university campus, only two were completely defoliated. Some were barely touched. Two months later they were completely refoliated. Any one seeking for parasites knows that usually only a small percentage of hosts are infected (15, ¶ 1). In a higher vertebrate, of forty-three parts liable to infection by more than that number of species of parasites, one usually finds a total of four or five species of non-protozoan parasites per individual. Many kinds can be harbored if the number of individuals of each kind are few. Of leaf miners only an occasional leaf is affected (with rare exceptions). What birds have developed the habit of feeding on leaf miner larvae? The great bulk of forest leaves are unmined. Other ex-

amples are numerous, there is ample food for every one
as long as numbers do not become excessive—and this
usually occurs only on the heels of man.   Even in
semiarid north China, quantities of grass on the hills are
left uneaten and are broken to dust by winter time and
swirled into high heaven by the fierce wind storms of
spring.   This problem of supply and numbers will be
equated eventually by the sociologist (certain ecologists).

Finally we are reduced to a consideration of birth rate
and here if anywhere is the crux of the matter.   Some
advantages may be secured by the new species:

   (1) In increase in number of eggs and/or number of
       broods.
   (2) In increase in fertility (per cent. of eggs hatching)
   (3) In increase of care of nest (decoy of eggs)
       (with or without increase in number)
   (4) In increase in care of young.

The first may be secured by increase in size of adult
or decrease in size of eggs.   The fourth may be secured
by increase in size of eggs with further internal develop-
ment of young.   The *possibilities are numerous.*   Any
change here gives the species a real interspecific advan-
tage or disadvantage.

There is no better example of this than among the
Anura of southern New England.   In this restricted area
there are ten species.   Among eight of these species there
is very close similarity and in some cases a pair are
separable only by close observation.   These couples are
Bufo lentiginosus and *B. fowleri, Hyla pickeringi* and
H. versicolor, Rana pipiens and *R. palustris, Rana clami-
tans* and *R. catesbiana.*   As already pointed out (16, p.
25), Europe has but one species for each of these pairs;
a similar condition obtains in north China.   Thus it
seems that northeastern America has nearly twice as
many Anurans as are to be found as a general thing in
the transitional zone.   This condition should bring on
''competition'' of an acute kind—if such exists.   To my
knowledge, no one has yet pointed out how the structural

differences between the two species of toads give the one an advantage over the other, nor how the specific differences between the four species of non-sylvan frogs (we have also a woodland species) give the one an advantage over another.

In the case of the toads, the only tangible differences are that *B. fowleri* breeds later (the breeding habitats are identical), and that it is more confined to sandy and drier localities (17, 18). This segregates *B. fowleri* to the sandy coast and river regions(18). Although there is overlapping of ranges the two rarely breed in the same ponds, neighboring ponds usually being occupied by one species or the other. "Competition," if it exists, should be easily observable and measurable in the pond areas where both species are found. When is or was this segregation established? Is psammophily due to a structural character (they barely differ structurally) or to a hormone, or is it "merely" habitudinal?

In the case of the Hylas the following biotic conditions exist:

	H. pickeringi (crucifer)	*H. versicolor*
Size	About 1 in.	2 in.
Size of habitus	Bushes, fences	On trees
Spring appearance	March 26	April 28 (at Boston)
Egg complement	1,000 (singly)	2,000 (small bunches)
Buoyancy	Demersal	Floating
Jelly envelopes	One	Two
Larval period	75–90 days	40–60 days
Size at transformation	Smaller	Larger

From this one would infer that *H. versicolor* would have several decided advantages. Yet it is recorded at Northampton, Massachusetts, as not rare (19) and *H. crucifer* as common. At Ithaca, New York, *H. versicolor* is less common (16, p. 2). On the whole there is no interference in breeding habits and probably not in niche occupied, but the latter should be carefully looked into.

The data for the four frogs follows:

	R. pipiens	*R. palustris*	*R. clamitans*	*R. catesbiana*
Size	3½ in.	2–3½ in.	3–5 in.	7–8 in.
Habitus	Meadows	Cooler waters	Meadows	Deeper water
Spring appearance	March 28	April 3	April 7	May 20
Egg complement	4,500	3,000	4,000	20,000
Buoyancy	Demersal	Demersal	Floating	Floating
Jelly envelopes	Two	Two	Two	One
Larval period	60–80 days	75–90 days	1 year	2 years
Size at transformation	Same	Same	Larger	Largest
Relative numbers	Most ab.	Less ab.	Solitary	Least common

As to habitus they all overlap and may all be found in the same pond. In fact one feels that their outstanding student (16) labors extremely to find a habitat difference. It can not be said that it is clear cut and definite. The egg buoyancy is correlated with season of appearance, namely after the last ice or frost, and secondarily, they get the full heat of the sun.

The most striking phenomena are: that the largest has by far the most eggs but is least common—undoubtedly due to its long larval period (a primitive condition—as well as the single jelly coat). Thus *R. catesbiana,* our largest frog, may be considered to be on the decline. By contrast, the most common species lays a fairly large egg complement, appears earlier than most of its enemies (water snakes, turtles, herons, etc.), has the shortest larval period and thus becomes amphibious and saltatorial earlier than any of the others. Here then are cardinal biotic factors which are actually equivalent to greater survival value.

In connection with the above, notice should be taken of the habitat "preference," namely that the big species take to big wood or big water, relegating the small species to what is left. Is this size sorting due to "combat" or is it merely mechanical, the mesh of the habitat acting as a sieve? Studies carried on in this region on Rana and Hyla should throw light on this problem.

Similarly, is *R. palustris* relegated to cooler waters by *R. pipiens* or does it take to cooler water through physiologic urge? Is its longer larval period due to cooler waters or is it so long irrespective of water temperature?

Vitality statistics (under natural conditions) on the eggs (aeration, per cent. fertilized, etc.) might throw more light on relative numbers. That feeding idiosyncrasies affect health has already been pointed out (20, p. 206) especially in the case of one individual whose lung cavities were almost filled by parasites, while others were comparatively free. If such habits become general in one species it would become markedly reduced in numbers.

Under this section one might also note, in the dinoflagellates (8, p. 24) "that the most highly specialized genera . . . and the most highly specialized species . . . are, in the main, relatively rare in individuals. The development of adaptive structures of a highly specialized type is not in many instances accompanied by a corresponding reproductive vigor or equal enlargement of survival value."

V. ORTHOGENESIS

I have recently reviewed a monograph (21) on a group of protista in which the writer repeatedly discusses orthogenesis within each genus, arranging his species according to this orthogenetic development or that. In a similar way I may arrange all the different types of bottles found in a Chinese junk shop in orthogenetic series—basing the series on size, degree of coloration, surface sculpture, or, were I a limnologist, I might consider them as single celled organisms falling into two series: (1) those with large bodies, (2) those with long necks. The orthogenetic climax of the first group would be those with the smallest neck and roundest bodies (greatest capacity versus surface), while in the second those with the longest, slenderest necks would be the climax forms as supplying greater floatability through

development of a spine-like process, or better controlling orientation in the medium, or anything else my imagination could devise that seems probable. Orthogenesis within the genus is purely imaginary, for biotic orthogenesis requires a time interval equivalent to family or even ordinal development to become established. If the elephant's trunk is an example of orthogenesis, one would not refer to the relative differences in length of trunk within the genus *Elephas* or *Mastodon* as even indicative of orthogenesis. It is by comparison with other genera or subfamilies of the Ungulates that one gets the perspective necessary to appreciate orthogenesis. The genus, as far as structure alone is concerned, is merely a group of combinations of similarly non-adaptive structures, a pushing out in all possible directions. No highway has yet been discovered or determined, much less established. It is not until these structures have been developed so far as to be of adaptive significance that they can be regarded as orthogenetic. The genus is heterogenetic. The proof of this statement is that in the above mentioned monograph (21), as in other similar systematic contributions, the genera are divided into subgenera, each of which has its own line of development. In some cases the number of species in the subgroup is as low as two with one more ''primitive'' than the other in one *character!* Worse yet, in at least one such genus a special group of heterogeneous forms consisting of extremely simple and extremely complex structures, and not fitting into the other subgroups, is made! In other words even this clever ''orthogeneticist'' finds numerous exceptions to his devious lines. Orthogenesis postulates one line only through an *order,* various structures being in harmony. Why not forego such an orthogenetic urge and regard the genus as an astral pattern?

* * * * * * * *

The genus thus becomes a producer and testing plant for its own produce, for the most ''efficient'' species (one or more) having certain specified (generic) char-

acters—all the producing and all the testing being congeneric, a self-sufficient plant. Within wide limits, the environment is not functional; these limits being so wide as to allow for a great deal of specific latitude and congeneric interplay.

To what extent these tested species interplay with the tested species of other genera of the same family or other families depends on how similar are feeding habits, niche habits, protective habits, reproductive habits and to what extent adaptive structures are developed. The last, in the writer's opinion, is still of rare value; while habits are of dominant value even in intergeneric competition.

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OBSERVATIONS ON THE MANNER OF CLASP-
ING THE HANDS AND FOLDING
THE ARMS

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LUTZ was the first to point out that individuals vary in the manner in which they clasp their hands. Thus, if individuals clasp their hands in such a manner that fingers of the right and left hands alternate, it will be found that approximately half of them will have the right thumb uppermost, whereas half will have the left thumb uppermost. Lutz failed to find any correlation between the manner of clasping the hands and right- or left-handedness. He did find, however, that there was a slight predominance of individuals with the right-handed hand-clasps in $R \times R$ matings, and a slight predominance of individuals with left-handed hand-clasps in $L \times L$ matings. This Lutz considered to be evidence that the manner of clasping the hands is a hereditary trait, although he could not state the exact mode of inheritance.

Downey studied the manner of clasping the hands in 1,040 men and 541 women. She found that 49 per cent. of the men and 54.2 per cent. of the women place the right thumb outside. She also found a certain tendency for left-handed people to place the left thumb uppermost when clasping the hands. Thus, of 571 right-handed men 51 per cent. had a right-handed clasp, whereas only 37.4 per cent. of 131 left-handed men had a right-handed clasp. Similarly, 56.2 per cent. of 338 right-handed women, whereas only 46.2 per cent. of 91 left-handed women placed the right thumb uppermost.

In 1930, I visited 131 families with 642 children, my main purpose at that time being to obtain blood for a study of the heredity of the agglutinogens M and N of Landsteiner and Levine. On most of these families,

observations were also made on the manner of clasping the hands, 120 families with 469 children being thus studied. In 105 families the manner of folding the arms was also observed. As a result of this investigation the following conclusions were reached:

(1) About 50 per cent. of individuals prefer to clasp their hands with the right thumb uppermost, whereas the remainder have the left thumb uppermost. (The actual percentages in a series of 709 individuals were 53.1 per cent. with right-handed hand-clasps and 46.9 per cent. with left-handed hand-clasps). The preference is a marked one, so that the alternative position feels uncomfortable. There are a few individuals, however, to whom the manner of clasping the hands is indifferent.

(2) Similarly, about 50 per cent. of individuals fold their arms with the right arm uppermost, whereas 50 per cent. prefer to have the left arm uppermost. (The actual percentages in a series of 595 individuals were 44.4 per cent. and 55.6 per cent., respectively.)

(3) The manner of folding the hands and arms are constant traits of each individual, as was shown by reexamination of 22 individuals after a period of 18 months.

(4) These traits are not inherited; neither are they associated with right- and left-handedness or sex.

(5) Marked preference with regard to the manner of crossing the knees does not exist.

(6) These observations would indicate that the manner of folding the hands and arms are habits formed by each individual early in life, which remain constant throughout life. The particular manner selected by each individual seems to be a matter of chance only.

In Table I is presented the results of observations on 120 families with 469 children with regard to the manner of clasping the hands. It may be seen that for the general population of 709 individuals, 53.1 per cent. have a right hand-clasp, and 46.9 per cent. have a left hand-clasp. By the χ^2 method of testing goodness of fit that

TABLE I

FAMILY DATA ON THE MANNER OF FOLDING THE HANDS

Father's hand clasp	Mother's hand clasp	Number of families	Children's hand clasp				Totals
			Right-handed clasp		Left-handed clasp		
			Male	Female	Male	Female	
Right	Right	36	34	42	32	34	142
Right	Left	32	30	32	30	20	112
Left	Right	29	30	39	24	29	122
Left	Left	23	22	20	28	23	93
Totals—Children			116	133	114	106	469
Parents			68	65	52	55	240
Grand Totals			382		327		709
Per cent.			53.1		46.9		

has been worked out by Pearson and Fisher, it is possible to determine whether or not the manner of clasping the hands has any correlation with sex, and whether or not it is hereditary. The lack of correlation with sex is proved in Table II, and the absence of heredity and

TABLE II

PROOF OF ABSENCE OF CORRELATION BETWEEN MANNER OF CLASPING THE HANDS AND SEX

		Right-handed clasp		Left-handed clasp		n	χ^2	P
		Male	Female	Male	Female			
Parents	Actual	68	65	52	55	2	0.57	0.75
	Calculated	64	64	56	56			
Children	Actual	116	133	114	106	2	0.87	0.60
	Calculated	122	127	108	112			

correlation with sex in Table III. The method of calculation is very simple. Thus, in Table II, the calculation of χ^2 for the children is as follows:

For the 230 male children, the expectancies of 53.1 per cent. right hand-clasps and 46.9 per cent. left hand-clasps correspond to the approximate values 122 and 108, respectively.

Similarly, the corresponding expectancies for the 239 female children are 127 and 112, respectively.

Therefore, since

$$\chi^2 = \Sigma \frac{(x - x_0)^2}{x_0}.$$

Or $\quad \chi^2 = \dfrac{(122 - 116)^2}{122} + \dfrac{(133 - 127)^2}{127} + \dfrac{(114 - 108)^2}{108} + \dfrac{(112 - 106)^2}{112}.$

$\chi^2 = 0.87.$

And $n = 2$.

Then, from the table in Fisher's book: $P = 0.60$.

TABLE III

PROOF OF NON-HEREDITY OF MANNER OF CLASPING HANDS, AND LACK OF CORRELATION WITH SEX

Cross		Children				n	χ^2	P
		Right-handed clasp		Left-handed clasp				
		Male	Female	Male	Female			
R × R	Actual	34	42	32	34	2	0.29	0.88
	Calculated	35	40	31	32			
R × L	Actual	30	32	30	20	2	1.85	0.40
	Calculated	33	28	27	24			
L × R	Actual	30	39	24	29	2	0.58	0.75
	Calculated	29	36	25	32			
L × L	Actual	22	20	28	23	2	2.85	0.25
	Calculated	27	23	23.	20			

TABLE IV

FAMILY DATA ON THE MANNER OF FOLDING THE ARMS

Father	Mother	Number of families	Children				Totals
			Right		Left		
			Male	Female	Male	Female	
Right	Right	25	14	17	26	24	81
Right	Left	29	24	28	31	23	106
Left	Right	15	15	14	21	24	74
Left	Left	34	26	32	37	33	128
Totals—Children			79	91	115	104	339
	Parents		54	40	49	53	206
Grand totals			264		331		595
Per cent.			44.4		55.6		

In Tables IV, V, and VI is shown in a similar manner

TABLE V

PROOF OF ABSENCE OF CORRELATION BETWEEN MANNER OF FOLDING
ARMS AND SEX

		Right arm upper-most		Left arm upper-most		n	χ^2	P
		Male	Female	Male	Female			
Parents	Actual	54	40	49	63	2	3.92	0.15
	Calculated	46	46	57	57			
Children	Actual	79	91	115	104	2	1.35	0.50
	Calculated	86	87	108	108			

TABLE VI

PROOF OF NON-HEREDITY OF MANNER OF FOLDING ARMS, AND LACK OF
CORRELATION WITH SEX

Cross		Children				n	χ^2	P
		Right arm upper-most		Left arm upper-most				
		Male	Female	Male	Female			
R × R	Actual	14	17	26	24	2	1.02	0.60
	Calculated	17	18	23	23			
R × L	Actual	24	28	31	23	2	1.93	0.40
	Calculated	24	23	31	28			
L × R	Actual	15	14	21	24	2	1.07	0.60
	Calculated	16	17	20	21			
L × L	Actual	26	32	37	33	2	0.81	0.65
	Calculated	28	29	35	36			

that no correlation exists between the manner of folding the arms and sex, and that there is no heredity of this trait.

There are four possible combinations of the manner of clasping the hands with the manner of folding the arms. Thus, individuals who clasp their hands with the right thumb uppermost may fold their arms with either the right or the left arm on top; and the same statement holds for individuals with a left hand-clasp. These four classes may be designated as RR, RL, LR, and LL, re-

spectively, where the first letter represents the type of hand-clasp, and the second letter the manner of folding the arms. Of 219 parents, there were 51 of type RR, 64 RL, 47 LR, and 57 LL. For the frequencies of right and left hand-clasps given in Table I, and the frequencies of right and left arm-folds given in Table IV, the expectancies are 51 RR, 65 RL, 45 LR, and 58 LL, if there is no correlation between the two traits. Therefore, $\chi^2 = 0.121$, and since n = 3, P = 0.99.

Twenty-two individuals were revisited after a period of 18 months. Not one of them showed any change in the manner of clasping the hands. Three individuals, however, did not show any particular preference in the manner of folding the arms, which they varied from time to time.

Thirty-four left-handed individuals were examined. Of these there were 5 RR, 7 RL, 12 LR, and 10 LL. When we compare these frequencies with the frequencies in the general population (51 RR, 64 RL, 47 LR, and 57 LL) we find $\chi^2 = 4.10$; and since n = 3, P = 0.25, so that there is no apparent correlation with right- or left-handedness.

SUMMARY AND CONCLUSIONS

A large series of families was studied with reference to the manner of clasping the hands and folding the arms. The results indicate that these traits are not hereditary, show no correlation with sex, handedness or one another. They are probably habits formed early in life, which then remain constant throughout life.

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SHORTER ARTICLES AND DISCUSSION

SHELL GROWTH IN THE PERIWINKLE, *LITTORINA LITOREA*

THE formula, $y = bx^k$, where y is a linear measurement or weight of a part and x that of the whole while b and k are constants, has been used by Huxley and his students (1927, 1931) and others to describe differential growth in a number of forms, including molluscs, crustaceans, insects and mammals. When k equals unity, the part bears a constant relation to the whole and its proportion is unchanged with increasing size. This type is termed by Huxley "isogonic growth." When k is greater or less than unity the part increases or decreases relatively with increased size of the whole. These situations he terms "positive" and "negative heterogony." The magnitude of the constants are influenced by the part and the organism investigated while b, in addition, is of course dependent on the unit of measure employed. Usually, in positively heterogonic growth, k is less than 2, although as in the case of the female pea-crab (*Pinnotheres pisum*) it may greatly exceed the figure (Huxley, 1931). The constant k, has been designated by Nomura (1926a) the "specific exponent," while he calls b the "local constant," since in the same species he found that it sometimes varied from locality to locality.

This general formula has also been utilized to describe accurately the weight-length relationship in several forms, especially fishes and molluscs. When thus employed the value of k is usually greater than 3 but less than 4 (*e. g.*, Clark, 1928, Galtsoff, 1931), although for some species of fishes the weight seems to increase directly as the cube of the length.

On September 21, 22 and 23, 1931, the authors collected a large series of the periwinkle, *Littorina litorea*, within an area of a few square rods in tide pools near Bar Harbor, Maine. This series ranged in length of shell from 5.82 mm to 31.6 mm, probably representing all classes but the very young animals. After the shells had been freed from their occupants, they were allowed to dry in the open air for several days before being measured and weighed. Two measurements were taken on each shell. The length was taken as the distance from the apex of

the spire to the most distant point on the outer lip. The diameter of the aperture was taken as the distance from the junction of the outer lip and the body whorl to the most distant point on the former. These measurements were taken with vernier calipers to the nearest .1 mm, except in the case of some of the smallest shells, which were measured with a bench micrometer to the nearest .01 mm. All shells were weighed to the nearest .01 gram.

Table I gives the distribution of the shells according to length, together with the mean weight and the mean diameter with their coefficients of variability, for each class.

TABLE I

BIOMETRICAL CONSTANTS OF *Littorina litorea* SHELLS

Length (mm)	No. of specimens	Mean weight (grams)	Coefficient of variation (per cent.)	Mean diameter of aperture (mm)	Coefficient of variation (per cent.)
4.0 – 5.9	1	.020		4.16	
6.0 – 7.9	6	.063		5.55	
8.0 – 9.9	11	.174 ± .011	29.89	7.14 ± .091	6.25
10.0 – 11.9	24	.271 ± .010	26.20	8.55 ± .077	6.51
12.0 – 13.9	20	.408 ± .011	18.38	9.74 ± .090	6.11
14.0 – 15.9	22	.745 ± .016	15.03	11.48 ± .061	3.68
16.0 – 17.9	38	1.021 ± .026	23.02	12.89 ± .085	6.00
18.0 – 19.9	37	1.637 ± .027	15.09	14.72 ± .067	4.12
20.0 – 21.9	46	2.154 ± .026	11.88	16.17 ± .071	4.41
22.0 – 23.9	74	2.831 ± .028	12.50	17.50 ± .054	3.93
24.0 – 25.9	52	3.394 ± .036	11.34	18.55 ± .054	3.09
26.0 – 27.9	24	4.217 ± .049	8.39	19.37 ± .064	2.40
28.0 – 29.9	3	5.317		21.03	
30.0 – 31.9	2	6.570		21.95	

Although there is a considerable variation in weight in each length class, the mean weight increases at a definite rate with increased length. When the logarithms of mean weight are plotted against the logarithms of length, as shown in Fig. 1, the result, with the exception of the first class which contains but a single individual, is approximately a straight line. The relationship between weight and length, therefore, can be expressed by the formula, $y = bx^k$. Determining the constant b and
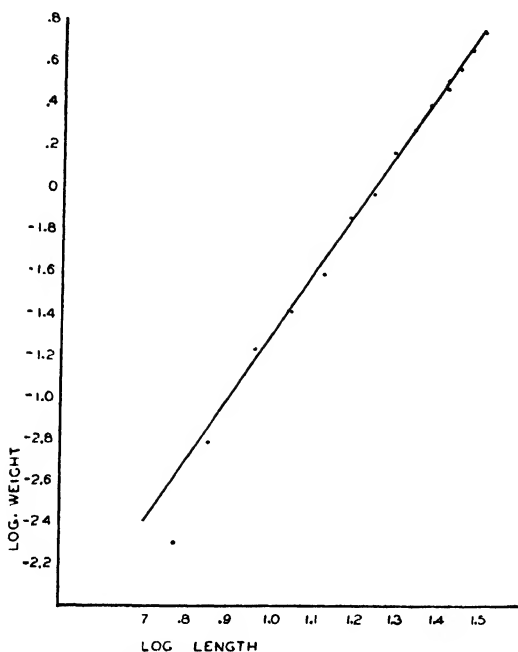
Fig. 1. Relationship between log. of weight and log. of length of shells.

k, by the method of least squares (omitting the two shortest and the two longest classes because of their small numbers), we obtain

$$\text{Weight} = .000194 \ \text{Length}^{3 \ 04160}$$

The curve of this equation is shown in Fig. 2, while the
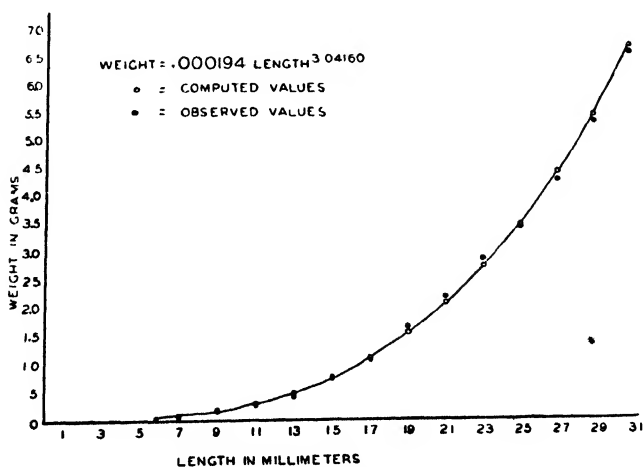
Fig. 2. Relationship between weight and length of shells.

calculated and observed weights are listed in Table II. It can be seen that weights computed from this formula fit the observed means very closely. Our value of k in the weight-length relationship of *Littorina litorea* shells is rather lower than that found for the bivalve, *Sphaerium heterodon,* by Nomura (1926a) or for the Hawaiian pearl oyster by Galtsoff (1931). In both these cases the value of the exponent was slightly in excess of 3.2.

TABLE II

OBSERVED AND CALCULATED VALUES OF THE SHELLS OF *Littorina litorea*

Length (mm)	No. of specimens	Observed mean weight (gms)	Calculated weight (ghs)	Difference between calculated and observed weight	Observed diameter of body whorl (mm)	Calculated diameter of aperture (mm)	Difference between calculated and observed diameter
4.0 – 5.9(5.82)	1	.020	.040	+ .020	4.16	4.42	+ .26
6.0 – 7.9	6	.063	.072	+ .009	5.55	5.32	– .23
8.0 – 9.9	11	.174	.155	– .019	7.14	6.84	– .30
10.0 – 11.9	24	.271	.285	+ .014	8.55	8.36	– .19
12.0 – 13.9	20	.408	.474	+ .066	9.74	9.89	+ .15
14.0 – 15.9	22	.745	.733	– .012	11.48	11.41	– .07
16.0 – 17.9	38	1.021	1.072	+ .051	12.89	12.93	+ .04
18.0 – 19.9	37	1.637	1.504	– .133	14.72	14.45	– .27
20.0 – 21.9	46	2.154	2.039	– .115	16.17	15.97	– .20
22.0 – 23.9	74	2.831	2.689	– .142	17.50	17.50	0
24.0 – 25.9	52	3.394	3.446	+ .052	18.55	19.02	+ .47
26.0 – 27.9	24	4.217	4.380	+ .163	19.37	20.54	+ 1.17
28.0 – 29.9	3	5.317	5.443	+ .126	21.03	22.06	+ 1.03
30.0 – 31.9	2	6.570	6.667	+ .097	21.95	23.59	+ 1.64

The relation between the diameter of the aperture and the length of the shell can likewise be expressed by the same general formula. Between the lengths of 8.0 mm and 27.9 mm inclusive we obtain the following formula by the method of least squares:

$$\text{Diameter} = .75940 \ \text{Length}^{1.00055}$$

This curve is depicted in Fig. 3, while the observed and calculated means are given in Table II. From this formula it is obvious that the growth in diameter of the aperture is approxi-
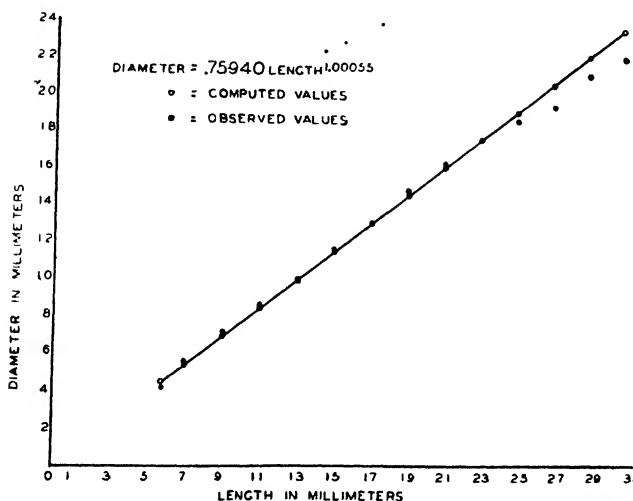
FIG. 3. Relationship between diameter or aperture and length of shells.

mately isogonic, although there seems to be a tendency for the computed diameters of the longest shells to exceed the observed. The two extreme classes, however, contain so few individuals that little reliance can be placed in them.

In most molluscan species which have been investigated linear relationships of the shells (height-width, width-length) are heterogonic, (Nomura, 1926, 1926a; Sasaki, 1926; Weymouth and McMillin, 1931) although in two gasteropods, Nomura (1926a) found the growth of height in relation to width to be isogonic.

The data on our series of shells of *Littorina litorea* show that the growth relation between weight and length can be expressed by the formula, $y = bx^k$, with the value of the exponent 3.0416.

It has also been determined that in our series the growth relation between the diameter of the aperture and the length of the shell is isogonic.

<div align="right">

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</div>

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VESTIGIAL CLAVICLES AND RUDIMENTARY SESAMOIDS[1]

Their Development and Functions in Mammals

It is interesting to observe the presence or absence of the clavicle, or collar-bone, in different groups of Mammalia, and the various stages to be found between a fully functional bone and a non-functional vestige.

While the clavicles in the Canidae have been known to anatomists, it is almost an unheard-of thing to find them in a mounted dog or wolf skeleton, yet it is safe to say that they are never absent in the living subject, although extremely small and quite without function. They have no articulation with any part of the skeleton, but are simply lodged among the muscles of the shoulders, there being no bone connection between the foreleg and the body. The scapula, or shoulder-blade, with which the foreleg articulates, is connected with the rest of the skeleton only by muscles and tendons.

[1] Photographs by the author.

In a large-sized dog skeleton, the Russian wolfhound, which has recently been mounted and placed on exhibition in the American Museum of Natural History, I find the clavicles roughly almond shaped, measuring 4 mm wide at their greatest vertical diameter, and 10 mm in length; while in a whippet, a small racing dog standing about 37 meters high at the shoulders, the clavicles measure only 2 by 3 mm.

It would appear that the Felidae must have found some use for this organ at a much later period than did the Canidae, as the cat family possesses quite a substantial looking clavicle, that of a lion being 7 mm in vertical diameter, and 73 mm long, a bone of sufficient size to demand the attention of the most careless observer, but one which is of very little, if any, service to its possessor at the present time.

A rather undersized raccoon skeleton, also on exhibition at the museum, shows a pair of clavicles which in form are curved shafts, measuring 1 by 8 mm. Here again we have vestiges handed down from the past, which have no function at the present time.

It will probably be found, upon investigation, that the clavicles are present in a more or less vestigial form in all the Carnivora.

The horses, asses and zebras (Equidae) and the cud-chewing animals (Ruminants) are without clavicles and so far as is known all the hoofed animals (Ungulates), but it does not necessarily follow that a remaining vestige may not yet be discovered in some species.

The clavicle is well developed and highly functional in man, in the bats (Cheiroptera), and is present in a functional form in most of the gnawing animals (Rodentia). It is well developed in all the pouched animals (Marsupialia) except the bandicoots (Paramelidae).

One of the important functions of the clavicle is to furnish a fulcrum upon which the pectoral muscles are exerted in effecting a strong movement of the manus, or hand, toward the median line, as is so characteristic in man, where this bone is still very strong and well developed. In this respect, the human subject has remained primitive. The massive pectoral muscle, with its origin on the sternum and insertion near the head of the humerus, and the reverse action of the trapezius, deltoid and infraspinatus muscles of the back and shoulder give the human arm a

great range of lateral movement. This is admirably illustrated when the lumberman in a northern camp drops his ax for a moment while he exercises his arms, thrashing his hands against his shoulders to drive the circulation and warmth to his cold finger tips.

In the Equidae, on the other hand, with their highly specialized limbs, where the action is restricted to a simple back-and-forth movement, there is not the same necessity for a clavicle.

In their evolution, the clavicles are of very early origin, and, in animals where they are still highly functional, their calcification begins at a surprisingly early period in the foetal development of the individual.

While, in a great many mammals, the clavicles are now in the last stage of a slow decline, the sesamoid bones, as a class, are in their early infancy and may be regarded as of comparatively recent origin. With the exception of the human subject, where there are but few sesamoids, they are very prevalent among the higher mammals. They occur less frequently in the more primitive mammals and are even fewer in number in the reptiles.

These bones are always formed in a tendon, from a single ossification center, at a point where a tendon passes over an articular surface where there is great strain, and violent action to be performed. The sesamoid, with its articular cartilage and lubricant, saves wear on the tendon and reduces friction, thus conserving power. The oldest and most highly developed and perhaps the most useful sesamoid in the system is the patella, or knee-cap, at the knee joint.

In most cases these bones appear to be speed adaptations, while in others they have been developed where a comparatively slow but strong movement is required, as for example in the aard-vark (*Orycteropus capensis*), an animal of no speed but most efficient in excavating great burrows in the ground. This peculiar South African animal has many highly developed sesamoids.

The Canidae are unusually prolific in the production of sesamoid bones, there being from sixty-two to seventy-eight and occasionally eighty-two. Many of these may not be considered as rudimentary, but are well developed. Eight are located at the knee joints, the others on the feet. On the palmar surface of the metapodials there is a set of forty of these bones. These are well developed and are present in most mammals.

The more rudimentary sesamoids are at the distal ends of the metapodials on the dorsal surface, as seen in the illustration on p. 1. Then, similarly situated on the distal ends of the proximal
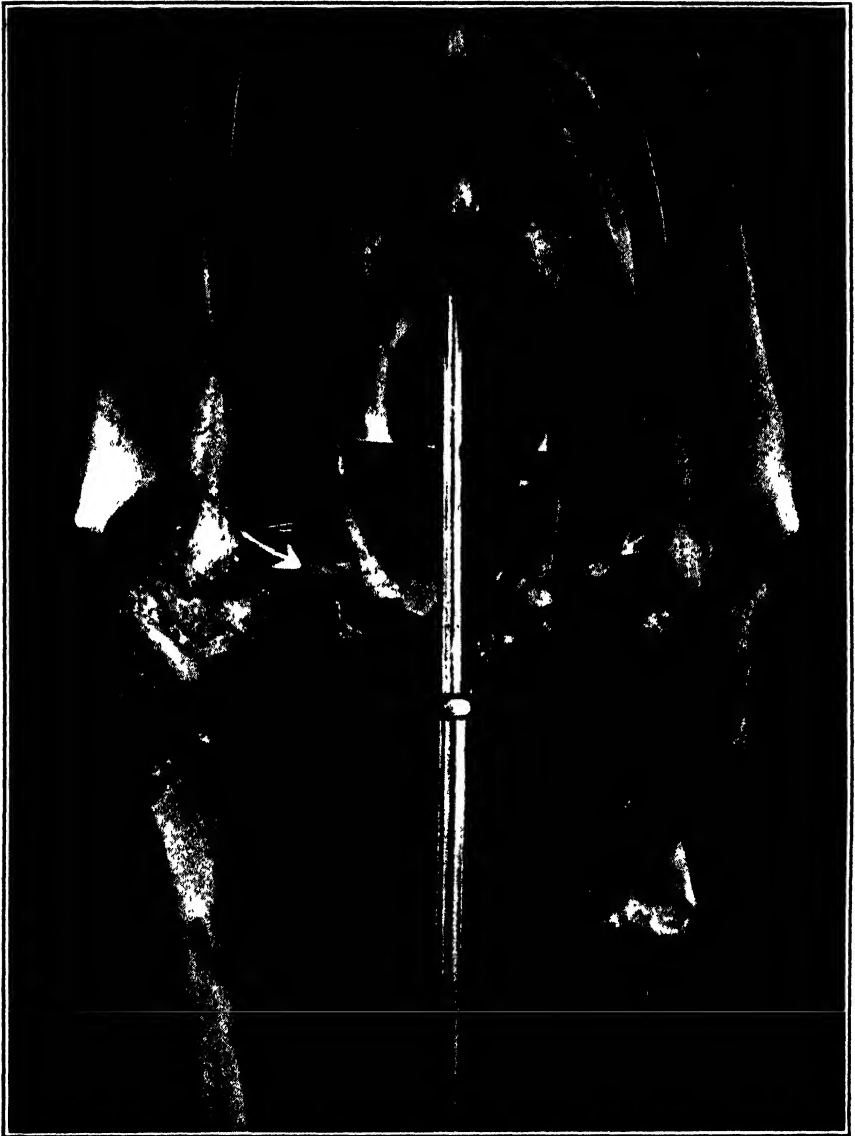
FIG. 1. Front view of a wolfhound's skeleton. The arrows point to very small vestigial clavicles.

phalanges, the middle joints of the toes, we find another set even more rudimentary and irregular in their occurrence. These of

the second set range from 3 mm to a size almost microscopic, or are frequently absent, so that it is not surprising that they have been so generally overlooked in the dissection and preparation of specimens. They are particularly interesting because they

Fig. 2. The bones of a wolfhound's foot. The arrows point to the rudimentary sesamoids.

are not generally known, although some of them were figured, by De Blainville, as long ago as 1839.

All these sesamoids that occur on the dorsal surface of the joints of the feet are, undoubtedly, of much more recent origin than any of the others and, we may assume, are continuing in their development, thus adding still further to the speed mechanism of these swift-footed animals.

Many of the sesamoids, particularly those that are more advanced in their development, are fairly well formed before birth; as it were, in anticipation of the hard work that they will be called upon to perform later, when their possessor is fleeing from a predaceous enemy, or is acting the part of the pursuer, or digging a hole in the ground to be used as he may find expedient.

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THE RÔLE OF MALE PARTHENOGENESIS IN THE EVOLUTION OF THE SOCIAL HYMENOPTERA

In the whole animal kingdom no other order can compare with the Hymenoptera in the number and diversity of its social species. In the whole animal kingdom, with a few notable exceptions, the production of males from unfertilized eggs is confined to the Hymenoptera. Is there an important causal relationship between these two facts? A consideration of the genetic consequences of male parthenogenesis, especially when acting in conjunction with another characteristic of most species of Hymenoptera, namely, the marriage flight, makes an affirmative answer to this question probable. Male parthenogenesis plus the marriage flight acts as an efficient eugenic contrivance ensuring the Hymenopteran colony a freedom from unfit individuals which is a marked advantage in competition with insect colonies of other species. Male parthenogenesis is a valuable adaptation to a social existence.

The marriage flight, besides its function of permitting crossing between colonies, serves as a selective contrivance by preventing unfit males from mating with the queen. Since the mating occurs high in the air, only sound males with good powers of flight can achieve it; individuals with defective wings or eyes, or any one of a variety of other possible hereditary defects, are eliminated as potential sires of the next generation of workers. A phenotypically sound father is thus guaranteed to the colony.

This alone, however, is not sufficient to ensure the transmission of a good heredity. It is only because the male is haploid that the selective action of the marriage flight is fully effective. In haploid individuals each chromosome and each gene is represented but once, not twice as in the more common diploid forms. Hence in the formation of germ cells there can be no segregation;

but one type of sperm is produced. The genotype is identical with the phenotype and every individual breeds as he appears. Thus the phenotypically sound male selected by the marriage flight to sire the next generation of workers can produce only genetically sound sperm. Moreover, since good traits are usually dominant, the workers in most cases will be without defects even when harmful mutations have segregated in the eggs produced by the queen. The selective action of the marriage flight is thus a good guarantee of physically sound progeny.

This high efficiency of selection characteristic of the Hymenoptera stands in marked contrast to the comparatively low effectiveness of selection in diploid species. Here a pair selected as phenotypically perfect may carry an indefinite number of harmful mutant genes capable of cropping out in their progeny. Congenitally defective young from healthy parents due to this cause are relatively common in most species of animals from Drosophila up to and including man. Probably even more common is the death of embryos due to the action of lethal genes.

Consider the consequences to, let us say, the honey bee, were both parents diploid and hence capable of transmitting harmful recessive factors. In many, perhaps in the majority of hives, one fourth or more of the workers would manifest some imperfection or die without emerging from the comb. This would mean at the least the waste of much valuable comb space, and might involve the support by the rest of the hive of defective individuals incapable of carrying their share of the hive's labors. Any colony thus affected would be at an obvious disadvantage in the struggle for existence. As the Hymenoptera are constituted, however, such a situation is practically impossible. The eugenic efficiency of their method of reproduction ensures a sound heredity for the great majority of colonies.

There is a second interesting genetic consequence of male parthenogenesis which may have played some rôle in the evolution of the Hymenoptera. This is the uniformity which it causes in all the diploid individuals of a colony. Since the male, being haploid, produces only one kind of spermatozoon, his daughters are identical with respect to at least half of their germ-plasm. They are not only all sisters, they are half way to being identical twins. As already pointed out, moreover, the traits of the male are more likely than not to be dominant, and this will further accentuate the uniformity of his progeny. The similarity of all

workers from one father may partly account for the ability of ants to recognize members of their own colony. Uniformity of instincts, too, may well be of value as a factor favorable to cooperative effort in a social existence.

While the method of sex determination and reproduction found in the Hymenoptera is perhaps as well adapted to the elimination of congenital defects as any that could be devised, there are yet certain conditions in which defective individuals may be expected to appear. Though it usually guarantees a good heredity for the females, it permits a high incidence of defectives among the males. If a queen is heterozygous for a harmful recessive gene, as must occasionally be the case, half her sons will be defective. The loss that might result from this has been met in the bees and ants by reducing the number of males and assigning them no part in the economy of the hive or colony. They are discarded after mating is accomplished. Besides the defective males, occasional defective females will probably appear. These may be due to three causes. First, a mutant spermatozoon may unite with an egg carrying the same mutation. This must be a very rare occurrence. Second, the queen may be heterozygous for a harmful gene that is not entirely recessive. Such genes, while not common, are by no means unknown in most species that have been studied. Should the queen carry such a one, it would reappear in half the workers. Third, there probably exist sex-limited mutations which do not express themselves in males, but only in workers or queens. A mutation of this type, acting on the maturation of the eggs, may have caused the few cases where a considerable proportion of the workers in a hive have been gynandromorphs.

Despite these loopholes through which imperfect germ-plasm can escape the action of selection, the manner of reproduction prevailing in the Hymenoptera stands unexcelled in its eugenic efficiency. Only among the termites has there been evolved a system that is at all comparable. In colonies of these remarkable insects the defective young are eaten by their more healthy brothers and sisters.[1] Thus a certain proportion of defective germ-plasm is continually eliminated, and no burden of termite incompetents is carried by the colony. Some waste is involved, however, in rearing the young to the point where defects appear;

[1] W. M. Wheeler, 1928. ''Foibles of Insects and Men,'' pp. 212–215. New York: Alfred A. Knopf.

that system is better in which no defective individuals can be born. It is a fact too striking to be a coincidence that the termites, which alone among insects can rival the Hymenoptera in the complexity of their societies, are, also like the Hymenoptera, endowed with a eugenic system of high efficiency. The Hymenoptera have the advantage, however, that they were endowed with their system from the start, for male parthenogenesis must have appeared very early in their evolution. This is not the only characteristic which peculiarly fits them to a social life,[2] but it is an important and unique one, and it may be regarded as among the major adaptations which have led to the development of so many social species in this order.

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[2] F. M. Wheeler, 1928. ''The Social Insects,'' p. 130. New York: Harcourt, Brace and Company.

THE
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CLONAL DIFFERENCES AND CLONAL CHANGES IN THE APHID *MACROSIPHUM SOLANIFOLII*[1]

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DURING the past few years five different parthenogenetic lines or clones of the aphid *Macrosiphum solanifolii*(= gei) have been bred in our laboratory in a study of various phases of the life cycle. Certain rather striking differences among them, and several remarkable changes in one of them, have been observed. These differences and changes throw some light on the contradictions and uncertainties which have characterized aphid experimentation, so that it seems desirable to put them on record for that reason, if for no other. Furthermore, they may eventually help to arrive at a better explanation of the physiological modification of genetic response in these insects.

THE CLONES INVOLVED

The five clones studied are in this paper referred to as A, B, C, D and E. A is a strain collected from a rose vine in Ann Arbor in April, 1923; it has been reared parthenogenetically ever since (though producing many gamic forms at intervals), has been the main source of experimental aphids for nine years, and is still in existence in a thriving condition. In November, 1929, it changed considerably in several respects, as described in

[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

the latter part of this paper; the modifications will be recognized by naming this clone A′ in all connections relating to these last two and one half years. Clone B was a green strain and clone C a pink strain, both collected near Woods Hole, Massachusetts, in July, 1928; both were brought to Ann Arbor where they were maintained for about two years, though the only experiments with C were conducted in Woods Hole in the summer of 1928. Clone D was a green strain and E was a pink one, both collected near Woods Hole in September, 1930; both were lost, the former after a little less, the latter after a little more, than a year in Ann Arbor.

It should be understood that very few of the comparisons to be made were the primary object of experiments. They are derived from numerous experiments aimed at other results. This accounts for the very unequal amounts of information available regarding the several clones, and for the fact that many of the contrasts are between the clones taken only two at a time.

Color of Females

The existence of two varieties of this species in which the parthenogenetic females are respectively pink and green has long been known; clones A(A′), B and D were green, clones C and E pink, in this sense. C and E were probably not quite alike in color, though no objective tests were made.

The principal other color difference was that B included both bright green and greenish yellow parthenogenetic females, with a number of intergradations between them. This difference was not usually distinct in the nymphal stages, and often became more and more accentuated during adult life; but the color was never, so far as was observed, reversed during the adult stage. A young adult that was yellowish often became more distinctly yellow as it grew older, but did not become distinctly green; and a young adult that was much brighter green than the average never became yellow later.

There appeared to be no genetic distinction between green and yellow females, for green females gave birth to both green and yellow daughters, and yellow females produced both green and yellow daughters, in about the same ratio.

These green and yellow individuals of clone B differed from one another in the production of winged offspring in response to light and darkness. The green ones produced more winged offspring than did the yellow ones, in both continuous light and in alternating light and darkness. The differences between the green and yellow females of clone B, both in their color and in wing-production in their offspring, have been described more fully in another paper (Shull, 1932), and the brief summary here given is designed merely to render complete in this article the account of the known contrasts among the five clones.

A difference in the color of the gamic females was noted in two of the clones, but no objective measure of it obtained. The gamic females of clone A were typically wax yellow (Ridgway's "Color Standards and Nomenclature") when obtained under conditions favoring gamic females, namely, low temperature and alternating light and darkness. Clone B produced only a few gamic females, which was the chief reason why a match of their color was not made and recorded. That color was always a pale yellow, and from memory I would now assign it approximately to Ridgway's massicot yellow, or perhaps colonial buff. Another clone, not one of the five chiefly referred to in this paper, which furnished the material for some of my earlier experiments, produced gamic females having a general green color suffused with spots of salmon-orange (Shull, 1925, p. 295).

COLOR OF MALES

The color of the males in clone C was never studied. That of the males of clone B is indicated, by a number of references in my notes, to have been a rather bright

red, a statement abundantly confirmed by memory. One of these references is to the fact that males in the general stock could hardly have been overlooked because of their unusually bright red color. The males of clones A′, D and E, however, were subjected to a careful analysis. Etherized individuals were matched with Ridgway colors, which were then recorded by name. Many male aphids were of nearly the same general color all over the dorsal surface, and these were recorded simply as of that color. Others were mottled, most often possessing a stripe down the middle line, a pair of poorly defined spots at the sides of this stripe in the anterior region, and another pair of spots in the postero-lateral regions. These several colors were separately recorded, with an estimate of the proportionate area covered by them. Subsequently these named colors were analyzed with respect to each of the elements entering into the Ridgway scheme of production, namely, (1) the percentages of the several spectral colors, corresponding in a general way to the wavelength, (2) the percentages of white and black and (3) the percentages of neutral gray. The numerical or other symbolic designations were weighted according to the areas covered, and the whole group of data statistically studied.

The percentages of the six spectral colors of the color wheel are indicated by numbers from 1 to 71. The mean colors of the three clones were found to have the following numerical values:

Clone A′	. .	11.82 ± .39
Clone D		16.20 ± .91
Clone E		9.91 ± .14

Each of these means is almost certainly significantly different from the others. Clone E, whose females were also pink, leaned most strongly to the red end of the spectrum; clone D was decidedly far toward the yellow. The standard deviations of these colors, and their coefficients of variation, were also determined. Only the latter need be given, as follows:

Clone A′	64.33 ± 2.35
Clone D	72.30 ± 3.98
Clone E	33.74 ± 0.99

The pink clone E had much more uniformly colored males than did either A′ or D. It is not quite certain that A′ and D differed significantly in the uniformity of male coloration, since their difference is less than twice its probable error.

The tints and shades of the colors, or percentages of white and black on the color wheel, are represented by Ridgway by letters. By reversing the order of the letters in the tints, the percentages of white and black may be thrown into a single series extending from white to black. The letters g to a represent decreasing percentages of white, while h to n extend the series through increasing percentages of black. These letters can not be converted into actual percentages for statistical study, since the differences in the percentages represented by adjoining letters are not everywhere the same. It is proposed, therefore, to regard the difference between adjoining letters as unity, regardless of the actual percentage differences of white or black represented by them. Also, numbers are temporarily substituted for the letters, with $f = 1$, $d = 3$, $b = 5$, full color $= 7$, $i = 9$, $k = 11$, $m = 13$. With these numerical values, the percentages of white and black in the males of the several clones are represented by the following means:

Clone A′	5.51 ± .13
Clone D	5.45 ± .27
Clone E	4.49 ± .06

All these colors are tints; there are no shades. Clones A′ and D are practically identical, with a mean tint value about half way between a and b, indicating about 7 per cent. of white. Clone E is significantly lighter than either A′ or D, since its mean tint is about half way between b and c, representing about 12 per cent. of white. The cofficients of variation in percentages of white and black were as follows:

Clone A′	43.12 ± 1.58
Clone D	64.66 ± 3.56
Clone E	29.95 ± 0.88

The pink strain (E) was the most uniform, clone D (a green-female strain) the most variable, with respect to the tint of the males. The differences in this regard were all plainly significant.

The broken colors in the Ridgway scheme are indicated by series of primes, ′ to ‴″, representing different percentages of neutral gray on the color wheel. In the calculations that follow, these primes are replaced by numbers 1 to 5. With this substitution, the amount of neutral gray in the mean colors of the males in the three strains becomes:

Clone A′	1.44 ± .05
Clone D	2.00 ± .07
Clone E	1.70 ± .02

Clone A′ exhibits the purest colors (about 43 per cent. of neutral gray), clone E the most broken (about 77 per cent. of neutral gray), the differences being almost certainly significant. The coefficients of variation of the percentages of neutral gray are:

Clone A′	56.30 ± 2.06
Clone D	46.45 ± 2.56
Clone E	27.04 ± 0.79

The pink strain E is most uniform, clone A′ the most variable, though the difference between A′ and D may not be quite certainly significant.

With the omission of the probable errors, these comparisons are collected in Table 1.

From this table can be read the following conclusions: (1) that, using the Ridgway names, the males of clone A′ varied around apricot buff as an approximate mean, those of clone D around cinnamon buff, and those of clone E around Japan rose; (2) that the males of the pink-female clone E were much more uniform in all respects than the males of either of the other clones; (3) that both of the green-female strains, A′ and D, varied

TABLE 1

COMPARISON OF THE COLORS OF THE MALES OF THREE CLONES WITH RESPECT
TO (1) THEIR WAVE-LENGTH, (2) AMOUNT OF WHITE AND (3)
AMOUNT OF NEUTRAL GRAY, ACCORDING TO
THE RIDGWAY SCHEME

Clone	Wave-length symbol		Amount of white		Amount of neutral gray	
	Mean	Coeff. var.	Mean	Coeff. var.	Mean	Coeff. var.
A′	11.82	64.33	5.51	43.12	1.44	56.30
D	16.20	72.30	5.45	64.66	2.00	46.45
E	9.91	33.74	4.49	29.95	1.70	27.04

greatly to left and to right (spectrum colors) in the Ridgway plates; (4) that males of clone D varied more than those of A′ in a vertical direction (amount of white) on the color plates; and (5) that males of clone A′ varied more than those of D in the amount of neutral gray.

NUMBER OF MALES

The only adequate test of the number of males produced by different clones simultaneously was made during the period from October, 1930, to March, 1931. The clones being reared at that time were A′, D and E, but these were repeatedly reared from wingless parents under the same conditions until large numbers of individuals were obtained. The numbers of males from the several clones is shown in Table 2.

The males are, under all conditions, distinctly more numerous in the pink-female strain E than in either of the green strains, and, under all conditions, slightly more numerous in strain A′ than in D. Incidentally, it appears in all clones that the light conditions do not influence male-production in any important way.

NUMBER OF GAMIC FEMALES

The most intensive comparison of clones with respect to numbers of gamic females was made by repeated experiments between October, 1930, and March, 1931. The

TABLE 2

SHOWING THE NUMBER OF MALES PRODUCED BY WINGLESS PARENTS IN THREE APHID CLONES SIMULTANEOUSLY REARED UNDER FOUR CONDITIONS OF LIGHT AND DARKNESS (ALL AT 18°–20° C.)

Clone	Continuous light			20 hours light 4 hours darkness			8 hours light 16 hours darkness			4 hours light 20 hours darkness		
	No. of ♀♀	No. of ♂♂	Per cent. of ♂♂	No. of ♀♀	No. of ♂♂	Per cent. of ♂♂	No. of ♀♀	No. of ♂♂	Per cent. of ♂♂	No. of ♀♀	No. of ♂♂	Per cent. of ♂♂
A'	4268	354	7.66	3334	244	6.82	4123	352	7.87	3062	183	5.64
D	3489	156	4.28	2994	175	5.52	3878	138	3.44	2867	79	2.68
E	2500	499	16.64	1817	244	11.84	2347	434	15.61	1700	233	12.05

TABLE 3

SHOWING THE NUMBER OF GAMIC FEMALES PRODUCED BY WINGLESS PARENTS IN THREE APHID CLONES SIMULTANEOUSLY REARED UNDER FOUR CONDITIONS OF LIGHT AND DARKNESS (ALL AT 18°–20° C.)

Clone	Continuous light			20 hours light 4 hours darkness			8 hours light 16 hours darkness			4 hours light 20 hours darkness		
	Total off-spring	No. of gamic	Per cent. of gamic	Total off-spring	No. of gamic	Per cent. of gamic	Total off-spring	No. of gamic	Per cent. of gamic	Total off-spring	No. of gamic	Per cent. of gamic
A'	4622	0	0.00	3578	6	0.17	4475	0	0.00	3245	0	0.00
D	3645	0	0.00	3169	0	0.00	4016	7	0.17	2946	2	0.07
E	2999	25	0.83	2061	27	1.31	2781	105	3.77	1933	99	5.12

clones reared at that time were A', D and E. They were
bred under four conditions of light and darkness, but all
at one temperature, namely, 18°–20°. Since in those
experiments only wingless parents were being used, and
since gamic females usually spring from winged parents,
the results here given do not indicate the propensity of
the several clones for producing gamic females, but only
the tendency to produce gamic females from wingless
parents. Significant clonal differences are, however, just
as likely to be demonstrated in this way as if winged
parents had been used. Table 3 gives the total numbers
of parthenogenetic and gamic offspring simultaneously
obtained from these three clones.

Clones A' and D exhibit the characteristic, long attrib-
uted to this and many other species of aphids, of produc-
ing almost no gamic females from wingless parents. The
not infrequent gamic females produced by wingless
mothers in the pink-female strain E is a striking excep-
tion to what was believed to be the rule.

Clone B was kept for about two years in a fairly ex-
tensive stock in which, though observations were fre-
quently made, gamic females were seldom found and
then only in small numbers. Both winged and wingless
females were present in numbers, so that, whichever type
of parent produced gamic daughters in this strain, gamic
females should have appeared if there was a tendency
to produce them. This stock was reared in a laboratory
which experienced the usual diurnal temperature changes,
conditions under which clone A produced gamic females
profusely at times. The few gamic females which were
produced by clone B appeared in "epidemics," but these
were not nearly so marked as those of clone A and were
separated by long intervals. Numerous experiments
were performed with clone B, but almost never simul-
taneously with clone A, so that no direct comparison can
be made. The only experiment in which gamic female
offspring were obtained was one in which the parents
were wingless, which might suggest that clone B re-

sembled clone E (Table 3) in this respect. However, small numbers of males without any gamic females appeared in a number of other experiments in which the parents were wingless; from this it appears likely that the production of gamic daughters by wingless mothers in the one experiment was not the usual occurrence.

Size of Tibiae of Gamic Females

It was generally recognized, even with the use of a hand lens, that the hind tibiae of the gamic females were stouter and darker in clone A than in clone B. No objective measurements of the color were made, but the sizes of the hind tibiae were measured in a small number of specimens with an ocular micrometer. In clone A the mean length was found to be 221 units of the ocular micrometer, the mean thickness at the widest point 9.5 units, or a thickness equal to 4.3 per cent. of the length. In clone B, the corresponding means were 280 and 8.0, or a thickness equal to 2.9 per cent. of the length.

The difference in length was presumably part of a general size difference between the two clones. Casual observation had indicated, before any measurements were made, that the females, both parthenogenetic and gamic, were larger in clone B than in clone A, but no measures of body size or of other parts were ever made.

The difference in length, thickness and color of the hind tibiae, together with the differences in body color between these same two strains (referred to in an earlier section of this paper), constituted a rather easy and certain means of recognition of the two clones.

Order of Occurrence of Males and Gamic Females

It has been stated in an earlier section of this paper that gamic forms usually appear in "epidemics." Several or many generations may pass with only parthenogenetic forms, and then gamic forms occur for several generations, sometimes profusely. In clone A it has been repeatedly observed that the gamic period has been in-

troduced by the appearance of males, the gamic females usually following soon thereafter. Since in this clone males are nearly always derived from wingless mothers, gamic females from winged mothers, such observations could be made only on general stocks where winged and wingless females were both present, or in experiments in which both types were being bred. Almost without exception, in both of these situations, the males have appeared first. The slight qualification indicated by "almost" in this statement is due to one or two occasions on which males and gamic females appeared practically simultaneously. In no instance did the gamic females appear distinctly earlier than the males. So regularly were the males produced first in these epidemics that, in the conduct of experiments with aphids intermediate between the parthenogenetic and gamic females, the occurrence of males was regarded as the signal to begin the treatment designed to produce the intermediate females. The gamic females seldom failed to appear later.

Only one of the other clones (B) was bred in such a way as to reveal the order in which the two sexes appeared in the gamic phase. This was shown first in a general stock which was under surveillance, in the first autumn after its collection at Woods Hole. Gamic females appeared in this stock in moderate numbers without being preceded by males. It is unlikely that the males were overlooked, for, as stated above, the males of clone B were exceptionally brightly colored. The same priority of gamic females in clone B was again shown a short time later (October, 1928) in an experiment with wingless parents. Gamic daughters were produced in the early or middle portions of the reproductive period, and then late in life a few males appeared from the same groups of wingless parents. Since there were 14 wingless parents, it is not proved that the same parents produced both gamic female and male offspring, but it is shown that the gamic females were produced before the

males. In the light of this experiment, it seems likely
that the early gamic females in the general stock referred
to above came from wingless mothers; but, as stated in
an earlier section, this unusual event was not repeated
in any later experiments with clone B. It is possible
that prior production of gamic females in this strain was
dependent upon some temporary reversal of the relation
of gamic forms to wings (or their absence) in the parents,
and was not an independent distinctive feature of clone B.

Wing Production in Response to Light and Darkness

The response of strain A to light and darkness, in the
production of winged offspring, has been extensively
studied, and fully described in earlier papers (Shull,
1928, 1929). The features of that response in relation
to which comparisons with other strains are now to be
made were the following. Aphids of clone A reared in
continuous light produced mostly wingless offspring;
those reared in alternating light and darkness produced
mostly winged offspring. This statement is true of both
winged and wingless parents, but the number of winged
offspring from winged parents was usually smaller than
from wingless parents under any given condition of light
and darkness. In order that light and darkness might
be very effective in producing winged offspring, the dark
period was necessarily at least 12 hours in duration. The
length of the light period was less important, but a 6-hour
light period was individually a little more effective than
any other, either shorter or longer, and very short
periods had much smaller effects.

The comparisons between strains, relative to the above
effects, are from two sources: (1) experiments in which
two or more strains were reared simultaneously under
the same conditions, (2) experiments in which a single
strain was reared under what were believed to be the
same conditions as those previously used for strain A.
Experiments of the former type were the less frequent,
because the investigation was not being aimed at such

comparisons, but they afford the most convincing evidence of a difference among strains. They are accordingly given first.

Direct Comparison of Strains. In the earlier years covered by this article, comparisons among two or more of the strains A, B and C were made twice. With all details omitted, and only the totals given, the results of these comparisons are shown in Table 4.

TABLE 4

COMPARISON OF THE RESPONSES OF WINGLESS APHIDS OF THREE CLONES TO
CONDITIONS OF LIGHT IN THE PRODUCTION OF WINGED OFFSPRING

Dates	Clone	Continuous light			16 hours darkness 8 hours light		
		Wing-less	Winged	Per cent. winged	Wing-less	Winged	Per cent. winged
Aug.–Oct., 1928	A	505	4	.8	103	265	72.0
	B	490	952	63.8	1070	172	13.8
	C	312	491	61.1	505	112	18.2
Dec., 1928	A	362	22	5.7	54	150	73.5
–Mar., 1929	B	177	233	56.8	166	37	18.2

It is shown in this table that clone A behaved as in the previously described work; that is, in continuous light it produced nearly all wingless offspring, while in alternating light and darkness a very considerable majority of the offspring were winged. Clones B and C, however, show a striking reversal of this relation, in that there were many more winged offspring in continuous light than in alternating light and darkness.

In the experiments of 1930 and 1931, the comparisons available are among clones A′, D and E. Here the numbers of individuals involved are large, coming from often repeated tests made largely for the purpose of ascertaining the differences among clones. The results of these tests, using alternating light and darkness of three different periods, are given in Table 5.

It is shown in this table (1) that under all conditions of light clone E produced fewer winged offspring than

TABLE 5

COMPARISON OF THE RESPONSES OF WINGLESS APHIDS OF THREE CLONES TO DIFFERENT CONDITIONS OF LIGHT, IN THE PRODUCTION OF WINGED OFFSPRING, OVER A PERIOD FROM OCTOBER, 1930, TO MARCH, 1931

Clone	Hours of light	Hours of darkness	Offspring		
			Wingless	Winged	Per cent. winged
A′	24	0	2395	1869	43.8
	20	4	1502	1819	54.7
	8	16	2063	1967	47.7
	4	20	1287	1734	56.6
D	24	0	2050	1437	41.2
	20	4	999	1993	66.6
	8	16	1780	2057	53.1
	4	20	1153	1702	59.4
E	24	0	2103	363	14.7
	20	4	1364	419	23.4
	8	16	1704	524	23.4
	4	20	1221	374	23.4

either A′ or D; (2) that each clone produced more winged offspring in alternating light and darkness than in continuous light, thus agreeing with clone A (though with much less marked differences) and disagreeing with clones B and C; and (3) that the duration of the periods of light and darkness made little difference in the number of winged offspring, quite in contrast with former results from clone A in which the dark periods must be at least 12 hours long to produce much effect.

Tests at Different Times but under Similar Conditions. The remaining comparison is that of clones B and A, derived from a series of experiments in which clone B was subjected to certain of the tests that had previously yielded striking results in clone A. It was impossible to carry on experiments as extensive as these on both clones simultaneously, so that direct comparison of the two clones can not be made. However, the conditions were kept as uniform and as nearly like those applied to clone A as possible. It is believed that the evi-

dence of a difference between the two clones thus obtained is valid.

One of the experiments on clone B was alternation of 6 hours of light with various periods of darkness. Clone A, under these conditions, had produced relatively few winged offspring when the dark periods were short, but as the duration of darkness approached 12 hours there was a very great increase in the proportion of winged individuals; and as the periods of darkness increased beyond 14 hours there was a gradual decrease in the number of winged offspring (Shull, 1929). The response of clone B to the same alternations of light and darkness is shown in Table 6. The females of this clone were of two different colors, green and yellowish, as described in another paper (Shull, 1932). These two types were separately tested in the experiments and their offspring are separately recorded in the table. Unfortunately the yellows can not be compared with the greens, because

TABLE 6

The Response of Green and Yellowish Females of Clone B to 6-hour Periods of Light Alternating with Various Periods of Darkness, in the Production of Winged Offspring

| Hours of light | Hours of darkness | Offspring | | | | | |
| | | Of green parents | | | Of yellowish parents | | |
		Wingless	Winged	Per cent. winged	Wingless	Winged	Per cent. winged
6	2	95	127	57.2	58	94	61.8
6	4	14	122	89.7	68	12	15.0
6	6	47	170	78.3	46	10	17.9
6	8	57	140	71.1	91	43	32.1
6	10	34	107	75.9	54	32	37.2
6	11	111	102	47.9	35	38	52.1
6	12	48	77	61.6	142	12	7.8
6	13	62	126	67.0	71	38	34.9
6	14	97	124	56.1	147	2	1.3
6	16	103	36	25.9	78	13	14.3
6	18	62	111	64.2	60	29	32.6
6	20	85	117	57.9	131	13	9.0
24	0	83	112	57.4	85	34	28.6

they were reared at different times, with lights of different intensities, and at somewhat different temperatures. The comparisons to be made are merely those among different periods of light and darkness as applied to the same kind of parents.

It is difficult to interpret the results in Table 6, owing to their irregularity. The winged offspring are not more numerous in continuous light (last line of table) than in alternating light and darkness, which is in disagreement with the results on the same clone (B) in Table 4, though it should be pointed out that the precise periods of light and darkness (8 hr.–16 hr.) used in Table 4 are not duplicated here. It is possible that the contradictory results in Tables 4 and 6 represent a real change in clone B, and that they should be included among the clonal changes in a later part of this paper.

Some of the irregularity of the percentages may be due to the rather small numbers of offspring obtained, and combining the data into larger groups might show a significant difference. Previous work on clone A suggests one way in which the data might be combined. In clone A, dark periods of less than 12 hours (alternated with 6 hours of light) yielded rather few winged offspring, while dark periods of 12 or more hours yielded many winged offspring. If the periods in Table 6 be grouped thus, the green parents are found to yield 68.2 per cent. of winged offspring for the shorter dark periods, and only 56.4 per cent. for the longer periods; while the yellowish parents yield 36.0 per cent. for the short periods and 16.7 per cent. for the long periods. From both kinds of parents, fewer winged offspring were produced with long dark periods than with short ones, which is just the reverse of the response formerly given by strain A.

A second test of clone B involved different durations of the period of light. Notwithstanding the discovery (in Table 6) that 12 hours of darkness was not of special significance for clone B, the experiment, first performed

on clone A, of alternating 12 hours of darkness with various periods of light was tried on clone B with the hope of finding a definite difference in the response. Only the yellowish type of parent was used in this test, the results of which are given in Table 7.

TABLE 7

THE RESPONSE OF YELLOWISH FEMALES OF CLONE B TO ALTERNATION OF 12 HOURS OF DARKNESS WITH VARIOUS PERIODS OF LIGHT, IN THE PRODUCTION OF WINGED OFFSPRING

Hours of light	Hours of darkness	Offspring		
		Wingless	Winged	Per cent. winged
1	12	86	128	59.8 ⎫
2	12	93	93	50.0 ⎬ 50.7
3	12	117	83	41.5 ⎭
4	12	95	90	48.6 ⎫
6	12	150	76	33.6 ⎪
8	12	101	42	29.4 ⎬ 38.0
10	12	92	104	53.1 ⎪
12	12	177	65	26.9 ⎭
16	12	105	66	38.6 ⎫
20	12	80	187	70.0 ⎪
24	12	106	186	63.7 ⎬ 59.5
28	12	82	110	57.3 ⎭
24	0	35	191	84.5

The proportions of winged offspring in this table are very irregular. Though this may be in part due to the small numbers of individuals reared, the difference between clones B and A can not thus be explained, since families no larger than these in the former work with clone A showed very distinct differences between the very short and very long light periods, on the one hand, and the intermediate light periods on the other. If the intermediate periods (say, 4 to 12 hours of light) in Table 7 be combined, and contrasted with the shorter (1- to 3-hour) and longer (16- to 28-hour) periods, the intermediate periods yield fewer winged offspring (38.0 per cent.) than either the short (50.7 per cent.) or the long periods (59.5 per cent.).

Continuous light in Table 7 yields more winged off-spring than any alternations of light and darkness, which is in agreement with clone B of Table 4. In Table 6, it will be recalled, clone B did not show any very significantly different response to continuous light as contrasted with the various alternations.

The third test applied to clone B, in comparing it with former work on clone A, was to rear it in alternating light and darkness in the ratio of 1 to 2, but with various actual durations. Clone A, under these circumstances, had produced many winged offspring when the dark periods were 12 to 20 hours long, fewer for the longer periods and very few for dark periods less than 11 hours (Shull, 1929). Clone B was bred from yellowish parents, with the results given in Table 8.

TABLE 8

COMPARISON OF THE RESPONSE OF YELLOWISH FEMALES OF CLONE B TO
LIGHT AND DARKNESS IN THE RATIO OF 1: 2, IN THE
PRODUCTION OF WINGED OFFSPRING

Hours of light	Hours of darkness	Offspring		
		Wingless	Winged	Per cent. winged
1	2	12	244	95.3
2	4	62	215	77.6
3	6	59	191	76.4
4	8	42	188	81.7
5	10	122	139	53.3
6	12	233	6	2.5
7	14	178	48	21.2
8	16	166	29	14.9
9	18	163	28	14.7
10	20	142	7	4.7
12	24	136	5	3.5
16	32	175	48	21.5
24	0	40	82	67.2

Despite the irregularities in the percentages in this table, the proportions of winged offspring are plainly greater for the short periods than for the long ones. Since in Table 7 it appeared that the duration of the

light was of minor significance, it seems probable that it is the duration of darkness which is of most importance in determining wing-production. If this conclusion is correct, it is the shorter dark periods, those less than 12 hours, which are most effective. Clone B is here sharply contrasted with clone A in which the dark periods of 12 to 16 hours' duration produced the most winged off-spring.

Summary of Wing Production. The salient features of the several clones with respect to wing production may be summarized in the following statements.

Clones B and C produced more winged offspring in continuous light than in alternating light (8 hours) and darkness (16 hours). In one test with clone B in which the alternating periods of light and darkness were other than 8 and 16 hours, respectively, there was no important difference between the response to continuous light and that in the alternations. These two results are probably contradictory, notwithstanding the difference between the actual duration of the periods. Clones A', D and E produced somewhat more, and clone A produced many more, winged offspring in alternating light and darkness than in continuous light.

When various dark periods were alternated with 6 hours of light, clone B produced more winged offspring for dark periods of less than 12 hours than for dark periods of more than 12 hours. This is the reverse of former results from clone A, which produced many more winged offspring for dark periods over 12 hours long than for dark periods less than 12 hours.

When various light periods were alternated with 12 hours of darkness, clone B yielded on the whole fewer winged offspring for the intermediate light periods (4 to 12 hours) than for either shorter or longer light periods. This is the reverse of former results with clone A, which yielded the maximum number of winged offspring at about 6 hours of light, fewer for both shorter and longer light periods.

When light and darkness were alternated in the ratio of 1 : 2, clone B produced more winged offspring for the short periods (up to 5 and 10 hours, respectively) than for the long periods. This is the reverse of former results on clone A.

Clone E produced fewer winged offspring under all conditions than did clones A' and D.

PROGENY OF WINGED *vs.* WINGLESS PARENTS

In three of the clones used in this work, winged and wingless parents were reared simultaneously on several occasions. The first comparison was made with clones B and C. Parents of both types were reared in continuous light and in alternating light (8 hours) and darkness (16 hours). The results are shown in Table 9.

TABLE 9

COMPARISON OF THE PROGENY OF WINGED AND WINGLESS PARENTS OF CLONES
B AND C, IN RESPONSE TO CONTINUOUS LIGHT AND
ALTERNATING LIGHT AND DARKNESS

Clone	Type of parent	Continuous light			16 hours darkness 8 hours light		
		Wingless	Winged	Per cent. winged	Wingless	Winged	Per cent. winged
B	Wingless	490	952	63.8	1070	172	13.8
	Winged	45	121	72.9	155	66	29.9
C	Wingless	312	491	61.1	505	112	18.2
	Winged	23	136	85.5	90	59	39.6

In both of these clones, more winged offspring were produced by winged parents than by wingless ones, both in continuous light and in alternating light and darkness. This is the reverse of the earlier results with clone A.

The next test of winged and wingless parents was in clone E. The two kinds were reared in continuous light and in three different alternations of light and darkness. Their progeny are shown in Table 10.

In clone E, more winged offspring were produced by wingless parents than by winged parents, under all

TABLE 10

COMPARISON OF THE OFFSPRING OF WINGED AND WINGLESS PARENTS OF
CLONE E IN RESPONSE TO SEVERAL CONDITIONS OF
LIGHT AND DARKNESS

Hours of light	Hours of darkness	Offspring					
		Of wingless parents			Of winged parents		
		Wingless	Winged	Per cent. Winged	Wingless	Winged	Per cent. Winged
24	0	510	87	14.6	308	0	0.0
20	4	276	133	32.5	311	46	12.9
8	16	452	30	6.2	305	19	5.9
4	20	382	95	19.9	213	7	3.2

conditions of light and darkness. This is in agreement with clone A, but contrary to the results with B and C (Table 9).

THE FREQUENCY OF WINGED-WINGLESS INTERMEDIATES

It was at once obvious that two of the clones differed in the frequency with which individuals having partially developed wings were produced. In one clone such intermediate individuals were very rare, in another rather common. So definite was the difference between them that, had the clones become accidentally mixed, the production of intermediates would, even in the absence of any other distinctions, have been relied on to effect a separation of them. Subsequent clones were found to range between these two in the frequency of their intermediates.

Comparison may first be made among all the clones used, by combining all the results of breeding under all sorts of conditions. The comparisons are not direct, for in most cases the clones were not simultaneously reared, but usually an attempt was made to keep the conditions constant, except as to light. In each clone, various periods of alternating light and darkness were used in different experiments, wingless parents were used in some and winged parents in other experiments, and the

temperature necessarily varied in different experiments. Nevertheless, the same types of experiments were performed in all the clones, in roughly the same proportions, so that clonal differences should still be conclusively shown in the total results. To separate out the experiments of each type would so reduce the numbers of individuals that the comparisons could easily be made less, rather than more, secure. Table 11 gives representative data, though by no means all that might have been included in clone A.

TABLE 11

Comparison of the Numbers of Intermediate-winged Females Produced by Several Aphid Clones. The Data are from Various Kinds of Experiments, most of them not Simultaneously Performed

Clone	Wingless	Winged	Intermediate	Per cent. intermediate
A	8592	7548	3	.018
A′	9104	9518	170	.905
B	10561	9232	360	1.786
C	1119	2246	2	.059
D	5982	7189	48	.363
E	8542	1925	40	.381

In view of the large numbers involved, the differences shown are certainly significant, since the clone with the greatest frequency of intermediates (B) produced 100 times as many, and several other clones produced 20 to 50 times as many, as the clone with the fewest intermediates (A).

From the data included in Table 11, a smaller number of individuals belonging to three of the clones may be used to make a direct comparison. These three clones were reared simultaneously, in four sets of controlled conditions of light, and at rather constant temperatures of 18°–20° C. These strictly comparable groups are segregated in Table 12.

Each clone produced more intermediates in alternating light and darkness than in continuous light; also, each produced more intermediates in the 8–16 hour alterna-

TABLE 12

COMPARISON OF THREE CLONES WITH RESPECT TO THE NUMBER OF INTER-
MEDIATE-WINGED FEMALES PRODUCED. THEY WERE REARED SIMUL-
TANEOUSLY AT TEMPERATURES OF 18°–20° C., AND IN THE
CONDITIONS OF LIGHT AND DARKNESS INDICATED

Hours of light	Hours of darkness	Clone	Wingless	Winged	Intermediate	Per cent. intermediate
		A′	1287	1734	41	1.34
4	20	D	1153	1702	10	.35
		E	1221	374	6	.37
		A′	2063	1967	93	2.26
8	16	D	1780	2057	34	.88
		E	1704	524	14	.62
		A′	1502	1819	7	.21
20	4	D	999	1993	2	.07
		E	1364	419	7	.39
		A′	2395	1869	4	.09
24	0	D	2050	1437	2	.06
		E	2103	363	9	.36

tion than in any other periods. Clone A′, however, showed very striking differences in its responses to the light conditions, clone D moderate differences, clone E only small differences.

Incidentally, it may be pointed out that in each clone the intermediates are least frequent in those conditions in which winged individuals are least frequent (continuous light), but that in general the intermediates are not most frequent in the conditions in which wings are most abundant. Clone E is somewhat exceptional in this regard, since the small fluctuation of its numbers of intermediates takes place along with almost exact uniformity of the number of winged individuals in the various periods of alternating light and darkness.

HEALTH IN ANN ARBOR AND WOODS HOLE

It has not been feasible to breed Woods Hole strains in Ann Arbor, nor the Ann Arbor strain in Woods Hole,

over long periods of time. Clone A, which was collected in Ann Arbor, was taken to Woods Hole in the summers of 1928 and 1929, but did not thrive. Experiments with it there yielded less than one fourth of the number of offspring obtained in Ann Arbor. Fewer offspring were produced, and many died. Dead bodies were often found overgrown with a fungus, but it is doubtful whether this was the chief cause of mortality. More than once it seemed likely that strain A would be lost there. In Ann Arbor this clone has been rather uniformly vigorous, the exceptions being readily traceable to unhealthy plants.

Clone B, which was collected in Woods Hole, throve exceedingly well there, and succeeded rather well in Ann Arbor for a year. During that year, however, it was subject to several attacks of some disease, presumably, which greatly reduced its numbers. Several experiments were lost because of these attacks. During its second year in Ann Arbor clone B suffered these attacks repeatedly. Almost no experimental results were obtained from it during that year, because experiments started during a period of apparent vigor were lost in the next depression, which was never long delayed. The clone was finally completely lost after about two years in Ann Arbor, despite every effort to maintain it.

Clone C succeeded a little better than clone B in Ann Arbor, but was likewise lost in an unhealthy period in the summer about two years after its importation. Clones D and E were used for experiments for about a year after being brought to Ann Arbor, but were lost in the summer at the end of that year.

Each of these strains was healthy and vigorous at home, but was maintained with difficulty when transported to another region. The reason is presumably climatic, but nothing is known concerning it. Woods Hole conditions were much more detrimental to the Ann Arbor strain than were the Ann Arbor conditions to the Woods Hole clones.

STRIKING CHANGE OF ONE CLONE

The Ann Arbor strain (A) underwent several remarkable changes in its behavior, chiefly with respect to wing-production in response to light and with respect to production of gamic daughters by winged females, after it had been reared in the laboratory for more than six years. These changes were concentrated in the fall of 1929, but one less striking change has probably occurred since then, and it is entirely possible that others have taken place without being discovered..

Production of Gamic Females. The sudden change in 1929 is best approached from the standpoint of the production of gamic daughters by winged mothers. Though clone A was brought into the laboratory in 1923, no winged individuals were used as parents in experiments during the first four years. Whether any winged parents produced only parthenogenetic daughters during that time is uncertain, though it would seem likely that they must have done so, since certain general stocks were not observed to include gamic females during the summers, and since in other strains previously used winged females had produced only parthenogenetic offspring at least during their first summer. When, however, winged females of clone A began to be used for breeding in 1927, they produced some gamic daughters in every such experiment, even in the summer. All families reared from winged females in 1928 and 1929 likewise included some gamic females, even in summer. In the summer and fall of the latter year attempts were made to induce winged mothers to produce only parthenogenetic offspring by rearing them at high temperature, which had been shown to favor parthenogenetic offspring (Shull, 1930a), in order to settle a certain question regarding the nature of gamic-parthenogenetic intermediates (Shull, 1930b). These attempts all failed, for all the groups of winged parents thus treated yielded some gamic daughters. It was thought at first that failure of high temperature to produce the expected wholly parthenogenetic families

might be due to the fact that the high temperature was applied only during the lifetime of the winged females, beginning soon after their birth. Consequently, a stock was reared at high temperature, so that the influence would continue generation after generation. During the early generations of this stock the temperature was kept at 24°, since earlier work (Shull, 1929) had indicated that no winged offspring would be produced at higher temperatures, and without winged females the purpose of the experiments would be defeated. When 24° was shown not to induce exclusively parthenogenetic offspring from winged mothers, the temperature was raised to 26° and then to 28°. Some winged females were still produced at these temperatures, and these females still produced some gamic daughters. Finally 30° was used, which in earlier tests had been shown to be nearly fatal when long continued; the aphids now endured this temperature, though producing only small families, and a few of their offspring were winged, and these winged females all produced some gamic as well as parthenogenetic daughters. The attempt to secure families consisting exclusively of parthenogenetic daughters from winged mothers had failed, and with it the attempt to determine the nature of the gamic-parthenogenetic intermediates likewise failed (Shull, 1930b, pp. 106–107).

The above narrative is designed to show, in the absence of direct experimental test, that clone A was experiencing a progressive change; that, probably over a period of years, its winged females were tending increasingly to the production of gamic daughters, even notwithstanding the deterrent influence of high temperature; and that, though this is of minor importance, winged females were being produced at high temperatures which formerly had entirely excluded them.

The offspring of the last winged females reared at 30° were placed at room temperature, as has regularly been done in these aphid experiments, to come to maturity. They were not examined until all were adult, and some

of their offspring were nearly half grown. It was then found that these latter offspring, instead of being green, were all of a straw-yellow color; and that, although their parents had been reared in continuous light which should (Shull, 1928) have made the offspring wingless, nearly all these yellow offspring were winged.

If something had happened which greatly increased the tendency to produce winged individuals, it was argued, it might now be an opportune time to start the old experiments anew, with a profusion of winged females at high temperature in order to get families that consisted only of parthenogenetic daughters. It was quickly found, however, that the high temperature was unnecessary. The daughters of these winged parents were all parthenogenetic without high temperature. This appeared to be the situation which was so long sought, in which the families of winged parents started as parthenogenetic. Attempts were promptly made to convert these families into gamic by rearing the winged mothers at low temperature, with the expectation that the desired intermediates between parthenogenetic and gamic would appear during the period of conversion. However, there was no conversion, for all the offspring were parthenogenetic. Alternation of light and darkness, which had been shown to favor gamic daughters, was added to the low temperature, but still no gamic daughters were obtained. Even when several successive generations of winged females were reared at low temperature and in alternating light and darkness, gamic offspring were not produced.

The transformation described in the second preceding paragraph occurred early in November, 1929. In two generations the winged members of clone A had changed from a condition in which they persisted in the production of some gamic offspring notwithstanding high temperature, to a condition in which they could not be induced to produce gamic daughters even at low temperature and alternating light and darkness. It was not until

December, 1930, thirteen months after this change, that gamic daughters were again obtained, even though low temperature and alternating light and darkness were repeatedly employed during that time.

· The yellow color which appeared in all the offspring in November, 1929, gradually disappeared over five or six generations. Since March, 1930, the normal green color has prevailed, though frequently healthy yellowish individuals have been produced, much as in clone B, as described in this paper and in an earlier article (Shull, 1932).

Wing Production in Response to Light and Darkness. The description of the response of clone A to light and darkness contained in my former papers (Shull, 1928, 1929) was based largely on experiments performed in 1927 and 1928. Somewhat the same behavior is known to have been exhibited earlier, though the experiments were then crude and the contrasts less sharply defined. That behavior, in the respects now to be used for comparison, was as follows. In continuous light, the offspring of wingless mothers were mostly wingless. In alternating light and darkness, the offspring were mostly winged if the dark periods were 12 to 18 hours long and the light periods 4 to 12 hours. Other periods of light and darkness showed various smaller effects, some of them practically no wing-production. The last adequate test of this behavior, before the reorganization in November, 1929, was made in November and December, 1928. Wing production at that time occurred essentially as described above.

Compare with this the behavior of clone A′ (the continuation of clone A after November, 1929) as shown in Table 12. The data concerning clone A′ in that table, with the omission of intermediates, are repeated in Table 13.

Although clone A′, in this table, is shown to produce fewer winged offspring in continuous light than in any alternating periods of light and darkness, the difference

TABLE 13

SHOWING THE RESPONSE OF WINGLESS FEMALES OF CLONE A′ TO LIGHT AND
DARKNESS IN THE PRODUCTION OF WINGED OFFSPRING,
FROM OCTOBER, 1930, TO MARCH, 1931

Hours of light	Hours of darkness	Offspring		
		Wingless	Winged	Per cent. winged
4	20	1287	1734	57.4
8	16	2063	1967	48.8
20	4	1502	1819	54.8
24	0	2395	1869	43.8

is not great. Clone A, in the former experiments, showed very few winged offspring in the last two lines of such a table, and fewer in the first line than in the second (*cf.* Shull, 1929, pp. 827–828). Compared with the earlier work on clone A, those of the winter of 1930–31 (clone A′) are indefinite.

What may be a further change in this clone is revealed by the experiments of the fall and winter of 1931–1932. From among more complete experiments, those portions which relate to wingless parents, and to the contrast of continuous light with alternating light and darkness, are selected for presentation here, because it is only these parts that afford any comparison with earlier results. These experiments, which were of several kinds, are collected into one group with reference solely to the difference in conditions of light, and their results given in Table 14.

In this table it is shown that fewer, instead of more, winged offspring were produced in alternating light and

TABLE 14

SHOWING THE RESPONSE OF WINGLESS FEMALES OF CLONE A′ TO LIGHT AND
DARKNESS IN THE PRODUCTION OF WINGED OFFSPRING,
FROM SEPTEMBER, 1931, TO JANUARY, 1932

Continuous light			8 hours light—16 hours darkness		
Wingless	Winged	Per cent. winged	Wingless	Winged	Per cent. winged
3531	4417	55.6	3969	2027	33.8

darkness than in continuous light, which is a reversal of the behavior prior to 1929. One feature of these most recent experiments which may render them invalid for the comparison just made is that the parents used were taken from stocks that had been kept at constant temperatures for more than a year, whereas the parents used in experiments prior to May, 1931, were usually kept at room temperatures from the first to the fourth instars (sometimes to early adult life). The two constant temperature stocks were kept at 24° and 14°, respectively. Parents from these two stocks did not differ greatly from each other, but both may have differed from parents kept at room temperature during their immature stages. There has been so far no opportunity of testing the possible effect of previous temperatures on subsequent offspring. Until such a test can be made it must remain an open question whether the lower percentage of winged offspring in the right half of Table 14 represents a reversal of earlier behavior of the same clone, or a response to a different set of conditions.

Frequency of Intermediate-Winged Individuals. Reference to the first two lines of Table 11 indicates that the Ann Arbor strain of aphids changed considerably in its tendency to produce partially winged females. Such intermediates were a rarity prior to 1929 (clone A); since that year fifty times as many intermediates have been produced (A'). The conditions under which A' produced these intermediates most freely have been partially analyzed in Table 12, where it is shown that alternating light (8 hours) and darkness (16 hours) favor their production. It is not feasible to analyze clone A in the same way because of the small number of intermediates obtained; but since alternation of light and darkness was very common in clone A, intermediates should have been produced by it if it possessed the property of doing so. This change, from rare to rather frequent production of intermediates, is one of the most striking observed in this clone.

WAS THERE INADVERTENT EXCHANGE OF CLONES?

It will be observed that clone A′ exhibits some of the characteristics of clone B. Clone B produced more winged offspring in continuous light than in alternating light and darkness; if the results in Table 14 are not due to antecedent temperature, clone A′ eventually came to respond in the same way. Clone B produced many yellowish females, so also does clone A′. Clone B produced many intermediate-winged females, so does clone A′.

It may occur to some that what happened in November, 1929, was not a change of clone A, but an inadvertent exchange of clones A and B. These two strains were in the laboratory at the same time, and such an exchange would have been physically possible. I believe, however, that the qualities of the strains themselves completely exclude the possibility of such an event, for, while clone A did change so as to resemble clone B in the several features named above, it retained its old characteristics otherwise.

In the first place, clone B, by the fall of 1929, had already suffered several epidemics of disease, while clone A had been uniformly healthy in Ann Arbor. After that there was still one strain subject to repeated epidemics, and one healthy strain. It seems scarcely likely that the diseased strain became healthy and the healthy strain diseased simultaneously in November, 1929. Furthermore, the response of the altered clone A (A′) to light and darkness in wing-production had not been completely reversed, but only rendered confused, by the winter of 1930–31, six months after the death of the clone designated B. Also, while clone B produced more winged offspring from winged parents than from wingless ones, clone A′, as shown by recent comparisons not included in this paper, is still producing generally fewer winged offspring from winged parents than from wingless. And lastly, the hind tibiae of the gamic females of clone A are still of the thickness and depth of brown color that characterized the old clone A. If, then, there

was an exchange of clones A and B in November, 1929, clone B changed then, or subsequently, in these four respects.

These facts, taken in connection with the yellow color and profuse wing-production observed at the time of the change, features that did not characterize either clone A or clone B, must be taken to mean that the two clones were not interchanged. Clone A must therefore have become modified in the several ways described in this paper.

Discussion

Nothing has been more characteristic of the investigation of life cycles of aphids than the general disagreement in the results obtained by different workers, even when using the same species and apparently the same environmental conditions. Varietal differences were of course to be expected on genetic grounds. It may well occasion considerable surprise, however, to find the differences as great as those described in this paper, even to the extent of complete reversal of some modes of reaction. In view of the differences here shown, it is not necessary to regard the early investigations as in any degree carelessly done or inaccurately described. While the lack of uniformity in the results obtained causes only confusion for the present and is to this extent regrettable, it is not improbable that, eventually, a better understanding of the physiology of genetic response in aphids will be attained because of these very discrepancies; for in the exceptions to a general rule is most often found the elucidation of the rule itself.

There may be an evolutionary significance of the results here described. The vigor of the several strains in their home locality, compared with their susceptibilities in other regions, suggests that in competition under natural conditions certain strains would be very promptly eliminated. Assuming a region in which gamic reproduction occurs periodically, one would expect stem mothers to give rise repeatedly to strains incapable of

enduring the prevailing conditions, as well as to strains more or less well fitted to those conditions. In regions where the winters are warm enough that partheno-genetic females may live over, which occurs in this species even as far north as Virginia (where gamic forms have not been found), changes of the sort that occurred in clone A in November, 1929, may be of significance. The change in clone A can not be regarded as a mutation in the ordinary sense because it presumably occurred in hundreds of individuals simultaneously. The changes produced were mostly physiological, though two of them resulted in visible differences, the production of yellow females and of intermediate-winged females. Whether these changes have a genetic foundation probably can not be tested in the usual way because of the extremely poor hatching quality of the fertilized eggs. From what is known regarding them, the modifications are probably to be regarded as physiological and entirely apart from the genetic mechanism as ordinarily conceived. The physiological changes are very interesting, however, from the fact that they persist over many generations with almost as great precision as mutations. Their mechanism can only be conjectured. One recalls the work of Ackerman (1926) on another species, from which he concluded that wing-production is associated with the dissolution of certain globules. It is difficult to think of such globules becoming cumulatively modified in a certain direction, suddenly becoming altered in a profound way, and maintaining the altered condition for many months in which thirty generations may have passed. Yet something has undergone just such a change in one of these clones. Whether changes of this nature can be of any evolutionary significance, even in warm regions where gamic reproduction is excluded, depends on whether the modifications are in any sense cyclical. If the aphids eventually return, by further changes of a similar nature, to their former condition, and perhaps repeat the whole performance, evolution would not get

far in this way. The fact that gamic females were again produced after an absence of thirteen months suggests such a cycle, but years of further study of the same clone will be required to demonstrate its existence.

✓Summary

Besides the long-known difference between pink and green varieties in this species of aphid, clones also differ in that some include yellow parthenogenetic females along with the green. The gamic females also differ in color in different clones. The colors of the males of different strains differ in wave-length, percentage of white and percentage of neutral gray.

A certain pink-female strain produced distinctly more males, and more gamic females from wingless parents, than did other strains reared at the same time.

Two strains differed markedly in the length, relative thickness and depth of color of the hind tibiae of the gamic females.

While males generally appear before gamic females in the onset of the gamic phase, one strain occasionally reversed that order.

While one strain produced very few winged offspring in continuous light and many in alternating light and darkness, three other clones reversed this relation. Several other related differences were found. One strain produced fewer winged offspring under all conditions than did any other strain.

Two clones produced more winged offspring from wingless parents than from winged parents, but two other clones definitely reversed this relation.

One clone produced nearly 2 per cent. of intermediate-winged individuals (more than 2 per cent. under certain conditions of light), others produced smaller numbers, while one produced only 3 intermediates in 16,000.

Each strain was healthy in the locality where it was collected, but was reared with difficulty when transported elsewhere (Woods Hole or Ann Arbor).

One of the clones changed suddenly in several respects. It suffered a great reduction in its tendency to produce gamic females; reversed its response to light and darkness with respect to wing-production; greatly increased its tendency to produce intermediate-winged females; began to produce numerous yellow individuals; and at the time of this great modification experienced a temporary change in the color (yellow) of all females and a temporary increase in the prevalence of winged individuals.

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TEMPERATURE AND OTHER FACTORS CONCERNED IN MALE BIPARENTALISM IN HABROBRACON

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In the parasitic wasp, *Habrobracon juglandis* (Ashmead) crosses of orange-eyed (recessive) females by wild type (black) males produce, according to sex-linkoid inheritance, black heterozygous females from fertilized eggs and orange azygous males from unfertilized eggs. In 1921 (Whiting, P. W., '21) black biparental males were also reported.

These anomalous males were called patroclinous because they showed the dominant paternal trait. They were supposed to be haploid mosaics with part of the body derived from the sperm nucleus alone (androgenesis). A few proved such by breeding test, but later genetic evidence indicated that the majority at least are diploid (Whiting, P. W., and Anna R. Whiting, '25). The genetic and chromosomal constitution of these males is of interest, bearing as it does on the problem of sex-determination in Hymenoptera. The ratios in which biparental males, females and haploid males occur has likewise excited interest. The two phases of the problem of male biparentalism, ratios and composition, have appeared somewhat separate, but doubtless each has an important relation to the other.

As a result of accumulation of several groups of data bearing especially on variation in ratios, it has been planned in the present paper to report such as seem likely to aid in solving the problem. The material is reported here for the first time except for that of Anna

R. Whiting ('24) which will now be briefly abstracted and resummarized in the light of later discoveries.

THE GENETIC FACTOR

It has been shown (Whiting, Anna R., '24) that certain crosses produce biparental males while others fail to produce them. Two groups of material, each inbred and descended from a different wild female, were designated as L from Lancaster, Pa., and I from Iowa City, Iowa. L contained both orange and black stocks while I was entirely black. Stock 12 was an orange-eyed stock of mixed L and I origin. The various crosses reported may all be summarized as in Table I.

It may be noted that percentage of males among the biparentals ($+ \male\male \times 100 / + \female\female \& + \male\male$) is relatively high when L is crossed with L; low when mixed stock, #12, is crossed either with L or with I; while if L females are crossed with unrelated I males, biparental males are altogether lacking. It was therefore suggested that crosses of related stocks would produce biparental males while crosses of unrelated stocks would fail to produce them. Thus they are unlike intersexes in Lymantria. This hypothesis has been substantiated by later results.

Magnhild M. Torvik ('31) bred several mutant types derived from different stocks up to L material and obtained biparental males in all cases. By the use of recessive mutations in two wild stocks (I and Minnesota) she identified biparental males thus proving that Lancaster material is not necessary for production of these males.

Crosses of recessive mutant type females by males from unrelated wild stocks from various sources have thus far failed to produce biparential males.

Referring again to Table I we may note that percentage of biparentals among total progeny ($+ \female\female \& + \male\male \times 100 / \text{Total}$) differs considerably in the different types of crosses. Crosses within the L material gave the lowest ratio of fertilized eggs reaching maturity; crosses

of unrelated stocks gave the highest, while the inbred stock of mixed origin by either L or I material gave an intermediate ratio. Percentage of biparentals appears then to be negatively correlated with percentage of males among the biparentals.

TABLE I

SUMMARY OF DATA ON PERCENTAGES OF MALES AMONG BIPARENTALS AND OF TOTAL BIPARENTALS FROM CROSSES OF RELATED AND UNRELATED STOCKS.
(ANNA R. WHITING, 1924)

Parents	Progeny				
♂ ♀♀×+♂♂	♀♀ +	♂♂ +	♂♂ o	$\frac{+♂♂ \times 100}{+♀♀\&+♂♂}$	$\frac{+♀♀\&+♂♂\times100}{\text{total}}$
L ♀♀ × L ♂♂ . .	4,364	332	7,272	7.07	39.24
L ♀♀ × I ♂♂	6,323	0	3,903	0.00	61.83
stock 12 ♀♀ × L ♂♂ .	1,547	47	1,361	2.95	53.94
stock 12 ♀♀ × I ♂♂	550	16	571	2.82	49.78

Data presented in Table I are based on insects reared at approximately uniform temperature, 30° C., so that variation in ratios due to that factor have been minimized. The significance of the exact ratios is, however, to some extent negatived by differences in length of life of the mothers. It has been shown by D. R. Charles ('30) that percentage of males among the biparentals decreases with increasing age of the mother (or with increasing time after mating). Charles reported the following percentages based on summaries from culture vials through which the mother was successively passed: (a) 11.96 ±.34; (b) 7.71 ±.57; (c) 7.77 ±.73; (d) 5.41 ±.75. As regards percentages of biparentals among the total there is likewise a complicating factor. Mated females produce both males and females, but after supply of sperm is exhausted males only are produced. Although difference in length of life of mothers is an important factor causing variation in ratios, and percentages of Table I are based on total progenies, the numbers presented here are large enough to be highly significant.

The facts are inconsistent with the theory that loss of a sex chromosome in gametogenesis causes males to develop

from fertilized eggs. Since males among biparentals are relatively frequent from L females by L males but are lacking from L females by I males, the loss would be in spermatogenesis of L males and if offspring from L females by I males be compared with those from stock 12 females by I males the loss must be in oögenesis of stock 12 females. Therefore if stock 12 females be crossed with L males the ratio of biparental males would be expected to be especially high, $7.07 + 2.82 - (.0707 \times .0282)$ per cent., instead of 2.95.

Another way of viewing the material is as follows. The difference between stock 12 females by L females and L females by L males must be due to greater loss in oögenesis of L females. L females by I males should then produce $(7.07 - 2.95$ per cent.$)$ 4.12 per cent., but not a single one occurred among 6,323 biparentals (females).

The conclusion seems inescapable that male biparentalism is dependent upon some condition at time of fertilization or subsequent to it.

Fecundity and Sex Ratio

Casual inspection of cultures gives the impression that unisexual (male) fraternities are in general larger than bisexual. In 1925–26 Arthur M. Cloudman reared over 5,300 wasps of inbred material (L stock 10, L and I stock 12). Transfers were made every four days and temperature, 30° C., and all other conditions were kept as uniform as possible. The mean number of offspring from the vials producing males and females was 13.69 while the vials producing males only averaged 18.05. The difference, 4.39 ± 1.30, is in favor of the unisexual. Female ratios were calculated for each of the "bisexual vials." The correlation between these ratios and numbers produced per vial was low but significantly negative: $r = -0.193 \pm .035$. Evidently other things being equal, the greater the proportion of females the smaller the total number.

TABLE II

FECUNDITY OF FEMALES OF VARIOUS TYPES

Females set Type	No.	Days	Eggs	Larvae	Cocoons	Pupae Naked viable	Pupae Naked lethal	Adults ♂♂	Adults ♀♀	Eggs/Days	Larvae×100/Eggs	Adults×100/larvae	♀♀×100/total
Daughters of biparental male	4	40	221	8	1					5.5+	3.6−	0	
+v $\frac{‡3}{‡11}$	2	17	365	301	245	7	1	252		21.5−	82.5−	83.7+	
‡3v	2	33	796	578	465	12	2	477		24.1+	72.6+	82.5+	
‡5v	3	53	907	569	506	10	1	486		17.1+	62.7+	85.4+	
‡5v × biparental male	2	40	480	356	315	6 ♂♂ 1 ♀	2 ♀ ♀	294	8*	12.0	74.2−	84.8+	2.6+
‡3 × ‡3 male	2	21	580	285	230	3	2	118	115	27.6+	49.1+	81.8−	49.4−
‡3 × ‡11 male	2	15	374	278	224	1 ♂ 2 ♀ ♀	1 ♂	41	177	27.6+	74.3+	77.7+	81.9−

* All tested and proved sterile.

In 1923 Anna R. Whiting carried out a series of observations on fecundity. Females of various types were set individually in small petri dishes containing a caterpillar previously stung by another wasp. Immobility was thus insured giving optimum conditions for egg laying. Observations were made daily on contents of all petri dishes and mothers were likewise transferred daily. Cultures were kept at 30° C. except for the brief time taken for observations.

Table II shows results as regards fecundity. The third column gives the sum of the days during which the females of a given type were set. This is equal to the total number of transfers made. The ratio, eggs/days (column 11), therefore represents egg production per day of each type of female; larvae/eggs expresses hatchability of eggs; and adults/larvae gives viability or capacity of larvae to develop to maturity.

Record is given (columns 6–8) of pupae formed, either in cocoons or naked. It may be noted that a rather high percentage of naked pupae are lethal while the majority of those forming cocoons eclose.

While it is true that ratios express characteristics of the progenies of but two to four females each, they may be taken as probably representative of the type as they are consistent with differences in general breeding tests.

As regards egg productivity, eggs/days, stock 5 appears to be somewhat below average and daughters of biparental males are very low. Eggs of the latter have little hatchability. Of the eight larvae produced, one was able to form a pupa, but this was lethal. Such a condition is typical for these females which are considered triploid.

Hatchability, larvae/eggs, of 80 per cent. or less and viability, adults/larvae, of 80 per cent. or more appear to be typical for progeny of virgin females and the same applies to females mated with biparental males. No reduction of number of offspring has ever been noted resulting from matings with biparental males. Very few

females are produced (here 2.6 per cent.) and it is probable that the sperm (diploid?) are few or are unable to penetrate the egg.

Marked decrease in hatchability of eggs (to 49.1 per cent.) may be noted in the two fraternities when stock 3 females were crossed with stock 3 males. This is in agreement with the observations of Cloudman that there are more offspring in male fraternities than in bisexual. Cloudman's bisexual fraternities came from sibling matings as in this case. Reduction in hatchability does not occur when similar females (stock 3) are crossed with unrelated males (stock 11), despite the high ratio of females produced (81.9 per cent).

We may note then that hatchability of unfertilized eggs is relatively high and viability of male larvae is good, although recessive lethals when present may be expected to kill fifty per cent of wasps in immature stages.

In outcrosses where female ratio is high (majority of eggs fertilized) and biparental males absent, hatchability and viability are little if any lower than in case of unfertilized eggs.

In case of inbreeding, where female ratio is relatively low and biparental males expected (although not identifiable in the present instance) hatchability is greatly reduced, although viability is normal.

DURATION OF DEVELOPMENTAL PERIOD

Observations of Anna R. Whiting above reported furnish evidence for time of development at 30° C. of types differing genetically. Time from setting of mothers to eclosion of offspring is given in Table III. Since this period is at least nine days and the mothers were transferred daily and records of eclosion were also made daily, the shortest period from oviposition to eclosion can not be less than eight days. A very few laggards and weaklings eclosing after eleven days are included in the eleven day group.

TABLE III

GENETIC DIFFERENCES IN DURATION OF DEVELOPMENT AT 30° C.

Females set		Progeny		Percentage eclosing after days as follows:		
Type	No.	Type	No.	9	10	11
+ v ♯3/♯11	2	+ & o ♂ ♂	252	29.4 −	49.6 +	21.0 +
♯3v	2	o ♂ ♂	477	10.0 +	59.7 +	30.2 −
♯5v	3	o ♂ ♂	486	7.8 +	58.6 +	33.5 +
♯5v × biparental ♂	2	o ♂ ♂	294	13.9 +	56.1 +	29.9 +
		triploid ♀ ♀	8	12.5	75.0	12.5
♯3 × ♯ 3 ♂	2	o ♂ ♂	118	5.1 −	47.5 −	47.5 −
		o ♀ ♀	115	0.9 −	36.5 +	62.6 +
♯3 × ♯11 ♂	2	o ♂ ♂	41	17.1 −	41.5 −	41.5 −
		+ ♀ ♀	177	9.0 +	46.9 −	44.1 −

Males from hybrid virgin females, + v ♯3/♯11, develop more rapidly than those from inbred stocks: ♯3v, ♯5v, ♯5v × biparental males. Females develop more slowly than males, and inbred females (from ♯3 × ♯3) more slowly than hybrid females (from ♯3 × ♯11). The eight triploid females from biparental males are too few for generalization, except to say that their period of development can not be very widely aberrant.

Sons of stock 3 females average the same rate of development whether the mothers are virgin or mated with stock 11 males. The latter cross produces no biparental males and, as noted above, fails to reduce fecundity of the females. Males are similar (from unfertilized eggs) in both cases.

If stock 3 females are mated with sibs, however, sons appear on the average to be delayed in eclosion. This may be interpreted as possibly due to the inclusion of unrecognized biparental males among them.

TEMPERATURE EFFECTS

At the suggestion of the senior author, the junior author carried out an experiment to test the influence of

various temperatures on ratios of males among biparentals and upon ratios of biparentals. Preliminary tests demonstrated that a temperature of 35° C. had a decided sterilizing effect on females and a lethal effect on offspring. Females reared at 30° C. were set at 35° and transfers were made every four days through vials a, b, c, and d. Of 40 cultures (single females) started, 37 contained eggs. Larvae appeared in only 24 vials from among which three defective males matured. Of 32 females passed through vials b only 17 produced eggs and none of these gave rise to larvae. The majority of these females were carried through vials c and d, but eggs appeared in only one case and these failed to hatch. Pans of water were kept at all times in the incubator and dead caterpillars became mouldy so that lethal and sterilizing effect may be attributed to temperature rather than to desiccation.

Orange-eyed females from inbred stock 3 were crossed with black-eyed males from related inbred stock 1. The mated females which had been reared at 30° C. were set either at 30° or at 20° and kept at those temperatures. Transfers of mothers were made when larvae appeared on the caterpillars. This occurred every four days at 30°, but at 20° larvae did not appear until ten days in vials a and until fourteen days after transferring to later vials. A generation was completed in approximately ten days at 30° while at 20° thirty-two days were required although adults appeared in vials a twenty-four days after setting. The longer time may have been due in part to delay in oviposition, but the shortened time of vials a may be attributed to accelerating effect of the higher temperature on the eggs before the mothers were mated. Larvae reared at the two temperatures were either kept at those temperatures or transferred to a different temperature when mothers were transferred to new vials. Transfers to lower temperatures delayed development as expected while transfers to higher accelerated it. The purpose of making the transfers was to

demonstrate any possible lethal influence which might affect the three classes of offspring differently.

TABLE IV

TEMPERATURE EFFECTS ON RATIOS IN PROGENIES OF ORANGE, ♯3, FEMALES CROSSED WITH RELATED TYPE MALES, ♯1. VIALS A & B

Temperature		Progeny				
Initial	After transfer	+ ♀ ♀	+ ♂ ♂	o ♂ ♂	$\dfrac{+ \text{♂ ♂} \times 100}{+ \text{♀ ♀ \& + ♂ ♂}}$	$\dfrac{+ \text{♀ ♀ \& + ♂ ♂} \times 100}{\text{Total}}$
30	30	233	21	251	8.268	50.297
	20	183	16	222	8.040	47.268
	35	331	36	436	9.809	45.704
	Total	**747**	**73**	**909**	**8.902**	**47.426**
20	20	362	10	234	2.688	61.386
	30	143	4	59	2.721	71.359
	35	221	0	64	0.0	77.544
	Total	**726**	**14**	**357**	**1.892**	**67.457**

Considering data from vials a and b (Table IV) we note a striking and significant difference in ratios for cultures started at the two temperatures. Difference in percentage of males among biparentals is $7.01 \pm .75$ in favor of those started at 30° while percentage of total biparentals is 20.03 ± 1.25 lower at 30°. It appears that lowered temperature may cause more eggs to be fertilized and of these a greater proportion produce females. Thus we have as a result of difference in temperature differences in these two percentages with negative correlation as has been shown also as a result of the genetic factor.

Differences are not significant among those started at 30° whether transfers were made to 20° or to 35°. Among those started at 20° an increase in percentage of diploids is noted when transfers were made to higher temperatures. The difference between those retained at 20° and those transferred to 35° is 16.16 ± 2.55 despite

a drop in percentage of males among biparentals, $2.69 \pm .45$. This may indicate a differential lethal effect of increased temperature upon males in general whether haploid or biparental.

TABLE V

TEMPERATURE EFFECTS ON RATIOS IN PROGENIES OF ORANGE, ♯3, FEMALES CROSSED WITH RELATED TYPE MALES, ♯1. VIALS C & D

| Temperature | | | | | Progeny | |
Initial	After transfer	+ ♀ ♀	+ ♂ ♂	o ♂ ♂	$\dfrac{+\ ♂\ ♂ \times 100}{+\ ♀\ ♀\ \&+\ ♂\ ♂}$	$\dfrac{+\ ♀\ ♀\ \&+\ ♂\ ♂ \times 100}{\text{Total}}$
30	30	46	4	139	8.000	26.455
	20	52	1	83	1.887	38.971
	35	142	8	446	5.333	25.168
Total		240	13	668	5.138	27.470
20	20	63	1	64	1.563	50.000
	30	86	0	38	0.0	69.355
	35	80	0	33	0.0	70.796
Total		229	1	135	0.435	63.014

Reference to Table V confirms conclusions drawn from Table IV. Percentages of males among biparentals are strikingly lower from older mothers (or older sperm) in agreement with findings of D. R. Charles ('30). Percentages of total biparentals are also lower, probably indicating exhaustion of supply of sperm. Those started at 30° have $4.70 \pm .98$ per cent. more males among the biparentals and $35.54 \pm .197$ per cent. fewer biparentals than those started at 20°. Differences among those started at 30° are of doubtful significance, but males are unquestionably reduced by transferring from 20° to higher temperatures as in case of results from younger mothers. No males appear among biparentals transferred from 20° to a higher temperature but nevertheless ratio of total biparentals is markedly increased. (Difference when transfers were made to 35° is 20.796 ± 4.148 per cent.)

SUMMARY AND GENERAL CONCLUSIONS

Crosses between related stocks produce biparental males while outcrossing fails to produce them. Ratios of males among biparental offspring are negatively correlated with ratios of total biparentals. Male biparentalism can not be explained by loss of a sex chromosome during gametogenesis, but, if such loss occurs, it must be at time of or subsequent to fertilization.

Daily observations of oviposition and offspring of a series of 17 females showed little variation in number of eggs laid per day according to type of female except that (triploid) daughters of biparental males were of low egg productivity. Hatchability of eggs and viability of offspring of triploid females is extremely low. Hatchability of eggs is to no great extent reduced by mating to biparental males (very few females produced) or by outcrossing to unrelated males (majority of offspring females), but if cross is made to closely related males (female ratio intermediate) hatchability is far below that of unfertilized eggs. Viability of offspring was neither decreased nor increased in comparison with that of male fraternities by mating with biparental, related, or unrelated males. These observations are in agreement with conclusions reached by extensive genetic tests. It is considered that fertilization by related sperm has a lethal effect perhaps by causing chromosome elimination.

The offspring (male) from the hybrid virgin females developed somewhat more rapidly than sons of inbred females. Females in general developed more slowly than males but hybrid females developed more rapidly than inbred. Brothers of inbred females appeared to be somewhat retarded in comparison with sons of similar mothers bred as virgins. It is suggested that this average retardation may be due to presence of biparental males.

Recessive females mated with dominant related males produce fewer biparental offspring but more males among the biparental if they are kept at 30° C. than if

they are kept at 20° C. Transfer of young to other temperatures appears to have no effect on ratios of the types except that ratio of biparentals is increased if transfers are made from low, 20°, to higher, 30° and 35°. This is probably to be explained by a differential lethal effect on males in general.

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SOME FACTORS AFFECTING THE REVERSAL OF SEX EXPRESSION IN THE TASSELS OF MAIZE

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INTRODUCTION

THE possibility of using the greenhouse in connection with corn breeding experiments was pointed out in 1921 (5). A crop has been grown each winter since then as a part of the corn investigations program at the Arlington Experiment Farm of the Bureau of Plant Industry. With better facilities during the later years it has been possible to examine some of the relations between environment and development under reasonably controlled conditions. Incidental to these, data were taken on the production of silks in the tassels of the different strains under different environments. These data form the basis for the present paper.

Maize normally is monoecious, with the pistillate inflorescences borne on lateral branches and the staminate inflorescence terminal. The flowers of both inflorescences are potentially hermaphroditic, however, with primordia of the organs of both sexes present (10). Moreover, pistils and anthers may develop in part or all of an inflorescence in which the opposite sex normally is expressed. Thus, in anther-ear and dwarf$_1$ (2), functional anthers develop along with the ovules of the ear shoot. Again, at least three recessive and two dominant genes are known that determine the production of functional pistils in the tassel (4). Considering only the latter, there are then definite hereditary strains of maize in which functional female flowers occur regularly in the terminal inflorescence under the same environment under which this inflorescence is male in most strains.

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It would seem that such strains constituted sufficient evidence of genic constitutions determining differences in sex expression in maize. In spite of this evidence, however, Schaffner concludes that "Any theory of sex which would explain the diversity of sexual expressions by an appeal to a diversity of gene constitutions is entirely beside the mark. Such conceptions and hypotheses are fundamentally incorrect because they do not agree with established facts . . ." (8, p. 285). Schaffner bases this conclusion on the results of his experiments (6, 7, 8) in some of which maize plants grown in the greenhouse during the winter in an environment of short days, low light intensity, normal growing temperatures, a productive soil and abundance of water produced tassels ranging from partially to completely pistillate. Schaffner terms the development of such tassels sex reversal and the term will be used in the same sense here.

Experiments at Arlington Farm, Virginia

The data to be reported were obtained on plants grown during the winters of 1929–30 and 1930–31. Three self-fertilized strains of corn were used in 1929–30, namely, 201-F, 228-4-8, and 616-4. No. 201 had been selfed for 11 generations and was a selection from the Delta variety, adapted to eastern Arkansas. No. 228 was a 10-generation self selected from the Lancaster Surecrop variety from Pennsylvania, and No. 616 was a 2-generation self selected from Polar dent, a variety developed at the Michigan Agricultural Experiment Station. In 1930–31 the same strains of 228 and 616 selfed for an additional generation were used.

The plants were grown in two units of the corn greenhouse. The scheduled temperatures for the "warm unit" were 80° F. in the daytime and 70° at night. The temperatures for the "cool unit" were 70° and 60°. There was no automatic temperature control, but these temperatures were reasonably approximated until blossoming was completed.

Two plantings were made each year, a first on November 15 and a second on December 15. From shortly after emergence until maturity some of the plants were given supplemental lighting from 60-watt mazda lights equipped with reflectors and suspended about six feet above the surface of the soil in which the plants were grown. The lights were spaced so that the average wattage was 3.2 per square foot.

In the 1929–30 experiments the lights were turned on from 4:30 to 9:00 P. M. each day, those plants not receiving the supplemental light being protected by black curtains drawn across the house. The same system was followed in 1930–31, and, in addition, some plants were given supplemental lighting from 7:30 A. M. until noon, thus increasing the light intensity instead of the light period. Lights of the same wattage and spacing were used to obtain the added intensity. The reflectors had to be tilted, however, so that the light was less concentrated than where the light period was lengthened.

TABLE I

PERCENTAGE OF TOTAL PLANTS HAVING SILKS IN THE TASSELS

Date planted and strain	Warm unit (80° F.)			Cool unit (70° F.)		
	Day-light only	Supplemental light		Day-light only	Supplemental light	
		Period 4:30–9 p. m.	Intensity 7:30 a. m.– Noon		Period 4:30–9 p. m.	Intensity 7:30 a. m.– Noon
Nov. 15, '29						
201	10	0		20	0	
228	0	0		30	0	
616	70	30		100	90	
Dec. 15, '29						
201	0	0		0	0	
228	0	0		0	0	
616	50	29		78	33	
Nov. 15, '30						
228	0	0	0	20	0	0
616	60	40	40	90	20	60
Dec. 15, '30						
228	0	0	0	0	0	0
616 ..	67	0	40	86	33	100

The numbers of plants with silks in the tassels in each experiment are shown as percentages of the total number of plants of the particular kind and treatment in Table I. Ten plants comprised a lot except for a few lots in which from one to three plants died. The average number of silks per reverted tassel in the 1930–31 experiment is shown in Table II.

TABLE II
AVERAGE NUMBER OF SILKS PER REVERTED TASSEL

Date planted and strain	Warm unit (80° F.)			Cool unit (70° F.)		
	Day-light only	Supplemental light		Day-light only	Supplemental light	
		Period 4:30–9 p. m.	Intensity 7:30 a. m.– Noon		Period 4:30–9 p. m.	Intensity 7:30 a. m.– Noon
Nov. 15, '30						
228			.	1		
616	36	19	19	42	48	73
Dec. 15, '30						
228				.		
616	47		40	47	22	91

ENVIRONMENT AND SEX EXPRESSION

That the length of the light period is important in determining sex expression, as already stressed by Schaffner, is well shown by the data in Table I. Of the 20 lots of plants receiving no supplemental light, 12 contained some plants having silks in the tassels. Each of the 12 comparable lots receiving a supplemental light period had fewer plants with sex reverted tassels. The number of silks per reverted tassel was not affected as strikingly, however, by lengthening the light period (Table II). Of the three possible comparisons the number of silks was less in two and more in one.

Of the 24 pairs comparable as to light but differing as to temperature, some plants in each of 13 pairs had sex reverted tassels (Table I). In 12 of these pairs the plants grown in the warm unit had fewer tassels containing silks than those grown in the cool unit, the relation

being the opposite in the other pair. The difference of some 10 degrees F. in temperature was essentially as consistently effective in modifying the percentage of sex reversals as was the difference of about 4½ hours of illumination. The number of silks per reverted tassel (Table II) was lower for the plants grown in the warm unit than for those in the cool unit in four of the five possible comparisons. Temperature, therefore, also was effective in this direction.

The percentages of sex reverted plants among the lots receiving supplemental light intensity differed very little from those of the comparable lots with no supplemental lighting (Table I). In three of the four comparisons there were slightly more sex reverted plants among the unlighted lots. The differences in numbers of silks per reverted tassel in the two lots (Table II) are entirely inconsistent. General observation of plants grown in darker and lighter greenhouses has suggested that low light intensity tends to sex reversal. If so, the differences in intensity in these experiments were too small to be significantly effective. It may be concluded, however, that the added intensity, which was something less than the intensity used to lengthen the daily light period, was not as effective as the lengthened light period in reducing sex reversal.

Detailed discussion of the influence on sex reversal of environment as modified by time of planting, either alone or in relation to the controlled features, seems unnecessary here. The plants of the December 15 plantings grew during days that were longer and in general had fewer sex reversals than those of the November 15 plantings. In addition, however, the light intensity for the December plantings was greater for conditions of equal cloudiness because of the sun being higher. Moreover, both of these differences were greater for the plants in the cool unit where development was slower than in the warm one, and this relation was modified further by interactions with the light treatments and with the time

required by the different strains to reach the blossoming stage.

For present purposes it is sufficient to have shown that with the same strain and the same temperature the length of the daily light period as controlled in these experiments determined, within limits, the percentage of reversals in sex expression. Similarly, with the same strain and the same length of daily light period differences in temperature determined, within about the same limits, the percentage of reversals in sex expression.

<center>HEREDITY AND SEX EXPRESSION</center>

Differences in the reversal of sex expression are at least as marked and consistent among the different strains as among the treatments.

Only five of the some 200 plants of strain 228 had silks in the tassels. All of these were grown in the November plantings in the cool unit without supplemental light. Three of them occurred in the 1929 planting and two in the 1930 planting, the latter having one silk in each tassel. There also was little reversal of sex expression in strain 201. In contrast, some of the plants in 19 of the 20 lots of strain 616 had reverted tassels, the average percentage being 56 per cent. in comparison with 2.5 per cent. for 228. Here, then, with a given temperature and light period, differences in heredity determined, within limits, differences in the reversal of sex expression.

Schaffner (8) also obtained a difference in the reversal of sex expression between two selfed lines. In his experiments the line with more sex reversals required longer from planting to blossoming. Schaffner assumed that excess of sex reversals in this line were due to the fact that the plants passed a longer time under short day conditions. This assumption would be more convincing were it not for the fact that this longer time carried the plants into a period of longer days. The average length of day from planting to blossoming accordingly was more for the line having more sex reversals. In our experiments it so happens that 616 requires the shortest

time from planting to blossoming and 201 the longest time, with 228 intermediate.

INHERITANCE OF TENDENCY TO SEX REVERSAL

In the December 15, 1929, planting in the cool unit there were no sex reversals among the plants of 228, whereas 78 per cent. of the plants of 616 had silks in the tassels. A plant of 616 with a high degree of sex reversal was selfed and crossed on a plant of 228. This cross was selfed and back crossed to the 616 parent in the field in 1930. Progenies of both parents, the F_1 cross, the F_2 and the back cross were grown in the corn breeding greenhouse at the Arlington Farm during the winter of 1930–31. The temperature was maintained at some 60° to 70° F. during the first weeks of development and then at some 70° to 80°. Electric lights were used to lengthen the daily light period in part of the house but not in the immediate vicinity of where these progenies were grown. Conditions, therefore, were moderately favorable for reversal in sex expression.

The proportions of plants in the segregating progenies having and not having silks in the tassels (Table III) are in good agreement with the expectation for an F_2 and back cross involving a difference in a single major factor pair, the recessive allelomorph determining the greater tendency toward sex reversal.

TABLE III

THE NUMBERS OF PLANTS WITH AND WITHOUT SILKS IN THE TASSELS IN
THE CROSS 228 × 616, ITS PARENTS AND IN SEGREGATING PROGENIES

Pedigree	Number of plants with		Percentage of reversals	Dev. P.E.
	Normal tassels	Silks in tassels		
228	6	0	0	
616	0	5	100	
228 × 616, F_1	9	0	0	
228 × 616, F_2	70	20	22.2	0.9
(228 × 616) × 616	52	49	48.8	.3

Ratios of normal to sex reverted plants other than 3 to 1 and 1 to 1 probably would have been obtained had the plants been grown in a different environment. Thus, with long hot days, presumably there would have been little or no reversal of sex expression even in the 616 parent. The evidence for a single gene difference is rather in the fact that (approximately) twice as many plants of the back cross had silks in the tassels as did those of the F_2 generation. This is true, moreover, not only of the totals but also of the more important classes for numbers of silks in the tassel as shown in Table IV.

TABLE IV

DISTRIBUTION OF PLANTS IN CLASSES FOR NUMBERS OF SILKS IN TASSELS

Classes for number of silks in tassels	Number of plants in class		
	616	F_2 cross	Back cross
1–4	1	14	27
5–8	1	3	5
9–12	2	0	1
13–16	0	1	3
17–20	1	1	1
More than 20	0	1[a]	12[b]

[a] 23 silks.
[b] Scattered, from 21 to 181 silks.

It seems reasonably conclusive that 228 and 616 differed with respect to an important gene determining differences in tendency to reversal of sex expression. The data of Table IV suggest also that additional less important genes also were involved in the cross, with 616 carrying those determining the greater tendency to sex reversal. The data are not sufficiently extensive to be conclusive on this point. The departure of the distributions from normal and the differences between the distributions, however, are indicative of such a situation.

DISCUSSION

The data reported here show that at least two elements of the environment, temperature and length of daily light

period, influence the reversal of sex expression in some strains of maize. Moreover, they show that the extent to which these environmental factors are influential differs markedly with strains of diverse heredity. Finally, they show that two of these strains differ by a single gene of major importance in determining their relative tendency toward reversal of sex expression.

The fact that the inherent differences caused by differences with respect to this gene require a certain environment for external expression is not unique. Emerson (3) has given a clear statement of the viewpoint of the geneticist with regard to sex determination in flowering plants. In this he emphasizes that many characters other than those associated with sex expression require both a specific genetic complex and a more or less specific environment for their expression.

Virescent seedlings in maize afford a good example of the interaction of genes and environment (1). Some of these are extremely albinistic and develop chlorophyll very slowly under any environment in which they have been grown. Others are virescent only when grown at relatively low temperatures or in subdued light or both. Under conditions more favorable for growth the latter can not be distinguished from their normal sibs. It is only natural that genes which produce a given end product under a wide range in environment should be used most by the geneticists. The geneticist recognizes clearly, however, that the other type of genes exists, and regularly uses a more or less controlled environment in working with such genes.

In fact, the complete acceptance of this principle as a matter of course by geneticists and a consequent failure to mention it may be a reason for the objections raised by non-geneticists to some genetic interpretations. Sharp (9) has summarized well what seem to be the major reasons for disagreement between the two schools, as follows:

1: A failure to recognize the real complexity of Mendelian Phenomena, and to understand what the geneticist should hold regarding genes and their action:

2: A failure to distinguish between the *segregation of genes* and the *differentiation of characters*, and hence to perceive how much is involved in the *determination of characters*: and

3: A failure to take proper account of the fact that the interacting system in which the phenomena under discussion occur is the system *organism-environment*, whose two components are not separable in any real sense.

Schaffner (8) appears to hold that before a gene can be said to determine a character it should control it completely in any environment. The other point of view, and one that to the writers seems more logical, is that evidence of genic control of a character in any single environment is an adequate basis for concluding that the differentiation of this character is determined genetically, with the full recognition that genes must always interact with environment in producing the final end results.

SUMMARY

Reversal of sex expression in maize, *i.e.*, the development of silks in the tassels, is shown to be influenced by environment and heredity.

Shorter daily light periods and lower temperatures tended to bring about the development of silks in the tassels, the opposite conditions tending toward normal sex expression.

The low additional intensity of light used during daylight in these experiments had little if any effect upon sex reversal.

Different inbred strains differed markedly in the degree to which they were affected by changes in environment.

The F_2 and back-cross progenies from a cross between two strains differing in their tendency toward reversal of sex expression segregated typically for a difference in a single major factor pair, the recessive determining the greater tendency to reversal. The data from these

progenies suggested that additional, less important genes also were involved.

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ARE THE CHROMOSOMES AGGREGATES OF GROUPS OF PHYSIOLOGICALLY INTER-DEPENDENT GENES?[1]

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IN current genetic theory the gene is regarded as a unit whose properties are inherent in its composition. The locus determines the manner in which the factor is distributed to the offspring with reference to the other genes in the complex. Physiological behavior, on the other hand, is a function of the make-up of the individual gene itself. Considering the genotype as a whole it is the combination, not the arrangement of the genes which determines the course of development. On this view the essential structure of the chromosome is comparable to that of a string of beads of diverse kinds. Each bead, corresponding to a gene, is a distinct entity and does not derive any of its properties from its neighbors. The inference is that the genes in a complement conceivably might be "restrung" at random without altering the manner in which they would function in development. It is assumed, of course, that in the permutation no genes are lost and none are added.

From time to time blocks of genes become shifted from one position in the chromosome complement to another. These changes sometimes occur spontaneously as Bridges ('23) first showed. They frequently result also from treatment with X-rays and radium (Muller, '28, Stadler, '28, and Dobzhansky, '30a). In maize several cases have been found in lines which have received no treatment calculated to produce germinal variations. The stability of the new arrangement appears to be comparable to that of the old. These changes open the way to a study of the

significance of the structure of the germ-plasm as such for heredity and evolution.

Structural changes in the chromosomes are of interest in two general respects. They may serve as physiological barriers to interbreeding between the type and the derivative form in which the position of certain germinal elements is different. Crosses between several strains of maize which breed true for new arrangements of parts of chromosomes and the normal type have been shown to give partially sterile hybrids. In one such combination studied, in which both ends of the *P–br* chromosome are replaced by segments from two other chromosomes, only about 15 to 20 per cent. of the pollen grains and ovules are functional. Furthermore, the materials are now in hand from which a true-breeding line might be synthesized which would probably be almost fully sterile with normal maize. It is apparent, therefore, that structural changes in the chromosomes may have the same potential significance as factors in the isolation of subraces of a species as have such well known physical barriers as mountains and large bodies of water.

Alterations in chromosome structure are likewise of interest as possible sources of changes in the dynamic properties of the gene complex. Will gene x, for example, have the same physiological effect in development if it lies between gene w and gene y (w-x-y) as it will if the x-y segment of the chromosome is transferred to a new position so that x now adjoins gene c (c-x-y)? In other words, are the properties of the hereditary units a function in part at least of the spatial relations which they bear to other genes in the complex? This is a problem of fundamental significance in heredity.

A remarkable feature of the homozygous translocated types of *Drosophila melanogaster* is that, in comparison with normal stocks of the same known genetic composition, they differ more or less strikingly in morphology and fertility. Few, indeed, have been obtained in homozygous condition so radical is the disturbance usually

attending the translocation. Bridges ('23) found the
"pale" translocation to be lethal in homozygous condi-
tion and accounts for the fact on the assumption that a
fragment of the translocated segment was lost in the
transfer. Of three translocations involving the third
and fourth chromosomes in *D. melanogaster* which
Dobzhansky ('30b) has studied in detail, one proved to
be lethal in homozygous condition; another, while normal
in appearance, was sterile in the female sex and showed
reduced fertility in the male; while in the third the
females produced markedly fewer offspring than the
wild type. Muller and Altenburg ('30) report that of
the various translocations involving the four chromo-
somes of *D. melanogaster* which they have investigated
only a few could be obtained in homozygous condition,
and that those which can be so bred usually show sterility
and different kinds of morphological abnormalities. In
explanation of this behavior it is suggested that accom-
panying a translocation a gene mutation may occur in
the chromosome involved or a deficiency may arise at the
point of break. It is possible, too, that the breaks may
occur where the chromosome has been injured previ-
ously. Muller and Altenburg also suggest that the
change in intermolecular surroundings of the genes ad-
jacent to the points of break and reattachment may alter
the way in which these genes function.

Semisterile–1 in maize which involves an interchange
of terminal segments between the *B–lg* and *P–br* chromo-
somes (Brink and Cooper, 1931) gives equal numbers of
semisterile and normal offspring on self-pollination.
Within the limits of random sampling one-half the latter
group consists of a new class of plants, termed $x-n_1$,
which is homozygous for the interchange. At the time
they were first obtained it was recognized that, in their
gross features at least, $x-n_1$ plants were phenotypically
indistinguishable from the standard, *o*–normal class. In
view of the significance of the question at issue, however,
it was deemed worth while to set up an experiment in

which $o-n$ and $x-n_1$ plants of the same average genetic composition could be tested more closely for possible developmental differences.

Progenies were grown from the seed of two selfed semisterile-1 sib plants not segregating for any known genes in the $B-lg$ and $P-br$ chromosomes. The semisterile plants among the offspring were discarded. The two classes of normals among the remainder were classified by mating each plant to an $o-$normal individual and examining the pollen of the respective hybrids the following year. In this type of mating $x-$normals give all semisteriles in contrast with the fully fertile offspring of $o-$normals.

The two classes of normals were necessarily distributed in the rows at random in planting. The following data were taken on them: (1) dry weight of the whole plant at maturity, (2) dry weight of the ripe ears, (3) number of days from planting to the silking stage. The latter figure is taken as a measure of rate of development. The results are summarized in Table I.

TABLE I

COMPARISON OF $o-$NORMAL (STANDARD) AND $x-$NORMAL (SEGMENTALLY INTERCHANGED) PLANTS OF THE SAME BREEDING

Character measured	$o-$normal		$x-$normal		Difference
	No. plants	Av. value	No. plants	Av. value	
Dry weight of plants (grams)	27	224.2 ± 14.8	30	233.7 ± 14.3	9.5 ± 20.59
Dry weight of ears (grams)	26	152.1 ± 13.0	29	153.2 ± 9.9	1.1 ± 16.30
Number days to silking	29	81.9 ± 0.35	32	80.28 ± 0.31	1.68 ± 0.46

It will be noted from the values presented in Table I that the differences between $o-$normals and $x-$normals, with respect to dry weight of plant and dry weight of ears, are clearly not significant statistically. In view of the rather large variability in these characters as indicated by the high probable errors larger numbers of in-

dividuals would have been desirable for the comparison. So far as the data go, however, there are no grounds for thinking that the interchange of chromosome segments has had any effect on size of plant or size of ear. The x-normal plants reached the silking stage 1.68 ± 0.46 days earlier, on the average, than the o-normals. The difference is 3.6 times its probable error and possibly significant statistically. It should be borne in mind, however, that in view of the relatively small numbers in the experiment the probable error of the difference is itself subject to a fairly large error of sampling and can not, therefore, be considered as certainly indicating a significant divergence in the two values.

The relations obtaining here are obviously different from those found by the Drosophila workers. The translocated type of maize is indistinguishable from the normal in fertility, in size of plant, in size of ear, and probably in rate of development.

In seeking an explanation of the difference the possibility should be kept in mind that, while in animals, gametes, which are more or less radically defective or otherwise unbalanced in their chromosome make-up, frequently function in fertilization, corresponding gametes in plants rarely or never occur on account of the incapacity of the gametophytes which might produce them to develop. The gametophyte generation, in other words, plays the rôle of a filter, intercepting such haploid combinations of the hereditary materials as radically modify the metabolic processes. It may be, therefore, that in plants one obtains in homozygous condition only a highly selected sample of the translocated types, namely, those which do not occasion any significant injury to the organism. In animals, on the other hand, degressive mutations, deletions, etc., which may be assumed frequently to accompany translocations are transmitted to the offspring along with the structural alteration.

There is an alternative explanation which should be tested if possible. It might be assumed that the chromo-

some in its essential make-up consists not of genes which are entirely distinct from each other in function but of aggregates of groups of genes which are physiologically interdependent. On this hypothesis it is supposed that propinquity of the genes within a group is essential to normal gene action. On this view translocations involving breaks between groups of genes would not alter the genotype. The case in maize discussed above might be assumed to be of this type. If, however, the chromosome is broken in such a way as to separate the members of a gene group more or less profound changes in the physiological properties of the complex would follow. All the translocations in Drosophila thus far described apparently would fall in this category.

Serious difficulties stand in the way of submitting this hypothesis to a genetic test. How is one to determine in a given translocation, for example, whether the position alone of certain genes has been altered? It would seem necessary to account for all the loci in which concomitant changes might have occurred, a condition which obviously can not be met. A cytological approach to the problem appears more hopeful.

Cytological and genetical observations are in agreement in showing that the chromosome is segmental in character even though the relations between the descriptive terms used in the two cases, chromomere and gene, are not yet apparent. Wenrich's ('16) studies on the grasshopper, *Phrynotettix magnus,* afford a convincing demonstration of the regularity in number, size and position of the chromomeres; and the breeding facts leave no room for doubt about the genes being arranged in a fixed succession. But this is as far as the substantial evidence goes. One can only speculate as to the probable relation between the elements into which the chromosomes have been resolved by the respective methods of the cytologist and the geneticist. It does not appear improbable, however, that the chromomeres (at least some of them) represent several genes. One need go but

a step farther and assume that the genes within a chromomere bear a special physiological relation to each other to form a consistent hypothesis as to why some translocations alter the reaction of the gene complex and others do not.   In the first class of cases it may be supposed that the break in the chromosome passes through a chromomere and in the second, where the structural change evokes no metabolic response, the chromosome is parted between chromomeres.

This is an hypothesis whose value can possibly be measured inductively by cytological means.   It was suggested by the observation that in early prophase figures of semisterile–1 maize one of the arms of the interchanged chromosome complex consists of three pairs of well-defined chromomeres. (Figure 3, Cooper and Brink, '31.)   One of the strands in this arm is a translocated segment, and the configuration shows that it corresponds to and is paired with three chromomeres of the normal chromosome.   Other preparations which Dr. Cooper has made show that these three chromatic bodies are a characteristic feature of semisterile–1.   Evidently the break in this chromosome occurred between chromomeres three and four (counting from the distal end).   There is likewise some evidence of a less certain character, however, that the other translocation in the complex involves a definite number of chromomeres and that the point of break lies between two of these bodies.   As discussed earlier in this paper plants which are homozygous for the semisterile–1 segmental interchange are readily obtained and do not differ significantly from normal maize of similar genic composition.   A study of other cases of translocations in organisms in which the details of chromosome structure at suitable stages can be made out should provide a body of evidence against which the hypothesis outlined may be tested.

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HOW OLD ARE THE LEPIDOPTERA?

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THERE is a general impression, based on the known geological record,[1] that the Lepidoptera are the youngest of the larger orders of insects. My feeling is that this is due merely to the imperfection of the geological record, and in particular to the fragility of the moths; and that the true origin of the Lepidoptera is ages older.

To extend back the hypothetical history of the moths to their origin we must consider how far back in the presumptive genealogical tree the known geological record goes, and figure the proportional time necessary to develop to that point from the bottom of the tree. We will also try to reconstruct the biological needs of the most primitive true butterfly and of the most primitive Lepidopter; and then discover in what geological periods they might have been supplied.

The conclusion that we will come to is that there is a slight weight of probability that the Lepidoptera arose in the late Carboniferous or early Permian period, in some part of the earth that was not glaciated, and that they arose in a sort of symbiosis with the first flowers; in fact, that the flowers were created by the Lepidoptera; and further, that the lower butterfly stage was reached in the Jurassic period.

The first point that we can fix is in the Miocene. Among the butterflies described from Florissant is one in beautiful preservation: *Prodryas persephone* Scudder. It was described as a rather distinct form, related to Libythea, but on examining the type I found that the venation has been misinterpreted, and that there was no significant difference in pattern, wing-form or visible

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[1] As, for instance, in Carpenter's recent article: "A Review of Our Present Knowledge of the Geological History of the Insects," *Psyche,* xxxvii, pp. 15–34, especially the diagram on p. 20.

venation from the modern Antillean and Neotropical genus *Hypanartia*. On the whole, the pattern suggests the African species sometimes separated as *Antanartia*, while the wing-form is nearest the wide-spread South American *H. lethe*. The differences in structure between the American and African types are obscure and entirely invisible in a fossil.

There was, then, in the Miocene period (some 20 million years ago by the radioactive dating) at least one modern tropical genus of a specialized family, already in existence. The other Lepidoptera from the Cenozoic age are less perfectly known, and none of them seem to give any more significant data, but none stand in contradiction.

Now we must look into the presumable ancestry of *Hypanartia;* and from this point back we must reconstruct the evolution of the order on the basis of living forms. The two families Lycaenidae and Erycinidae do not appear to be in the direct ancestry of the Lepidoptera, but if we leave aside a few characters presumably of modern origin (such as the abortion of the tegumen), we may say that the Pieridae lie below the Nymphalidae; below them, and more primitive in numerous structures of the body, wings and early stages, come the Swallowtails (Papilionidae). We can fairly say that the differences between the most specialized modern Nymphalidae and such a moderately generalized form as *Hypanartia* are small compared with the space that lies between *Hypanartia* and the *Papilios*. Here again we must pass over a few characters of the modern form (such as the scenthorns of the larva and the free vein 3rd A of the butterfly's fore wing), but it is safe to say that *Papilio* is not far from the earliest true butterfly.

The most significant feature of *Papilio* for its placing in the geological series is the food of the larva. The genus is divided into three main sections, evidently separated a long time ago. Two of these are so closely limited to single families of plants that we can safely

say that their ancestor fed on plants of those families: namely, the bird-wing butterflies and green-swallowtail group on the Aristolochiaceae, and the kite-swallowtails, including our Zebra, on the Anonaceae. The third group is more varied in food, but on the whole the oldest new-world subgroup is that of the Blue Swallowtail, *Papilio troilus,* and the oldest type in the old world, if we leave out of account the effect of mimicry on the pattern, is the one containing *P. clytia* and *paradoxa.* Both of these groups feed on the Lauraceae and the former on the Magnoliaceae also. The only other plant family which is a sufficiently dominant one in the list of food-plants for serious consideration is the Rutaceae (orange family). I am inclined to view this food-plant as secondary, like the Umbelliferae, Compositae, Rosaceae, Piperaceae, etc., which occur as casual foods for odd species or small groups. We find that these four families (Magnoliaceae, Lauraceae, Anonaceae and Aristolochiaceae) are generally conceded to be among the most primitive of all the flowering plants, some Magnoliaceae in particular having a specially primitive type of flower.[2]

A second factor of the picture is the biology of the genus. If we study the subfamily Papilioninae, we find that it is overwhelmingly of tropical and forest distribution, and that it tends to move out into the temperate zone in forested areas, but that only a few and rather widely isolated species (*e.g., turnus, philenor, marcellus, machaon* and *xuthus*) have really well-marked seasonal forms, and only a moderate number even have adaptations to a winter or dry season, most of them seeming to breed continuously. The number adapted to arid condi-

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[2] Here and throughout the paper I am using the term ''flower'' in a biological rather than a morphological sense, that is for a structure of whatever morphology which contains the reproductive organs, and associated display-structures which might well be for the attraction or convenience of insect fertilizers. The flower of such a primitive form as Lyginopteris will be parallel rather than identical with the modern Angiospermous type, and contrariwise the morphological ''flower'' of Lemna is hardly a flower from the point of view of the insect.

tions are even fewer and mostly belong to the rather specialized *machaon* group (feeding on Umbelliferae) or are outlying members of the Aristolochia group, such as *Euryades*.

On the whole, this seems to indicate that the Papilionidae were developed at a time when these four most primitive families of Angiosperms were among the dominant plants, when there was sufficiently high temperature and but little seasonal change.

Turning to paleobotany, we find that in the Cretaceous many of our modern temperate families were already dominant, and the climate was already seasonal, with essentially the characteristics of the modern temperate zone. The indication would be, then, that we must seek a somewhat earlier period, and one with a more consistently even and humid climate. This seems to point rather plainly to the Jurassic as the time of origin of the Papilionidae. Perhaps some botanist can suggest why the typical group of *Papilio* so early and so overwhelmingly switched to the Aurantiaceae. I suspect that it was due to a similarity of flavor combined with a reasonable degree of dominance of the family in the ecological pattern. Another point to be explained is why the outlying genera, many of them specialized for special environments, or with relatively small ranges, such as *Thais, Armandia, Parnassius, Eurycus* and *Sericinus*, are either feeders on the Aristolochiaceae, or obviously related to forms that feed on that family. It looks as though that family may have been very early in invading new types of climate.

Having attached the Papilionidae to the Jurassic period we must consider what lay before them in the history of the order. We can no longer hope for clear indications from food plants, as we are now back to the periods when almost all the families of plants are extinct ones, and the few that have had a continuous existence are forms largely shunned by plant-eating insects. We must assume that until the Jurassic the Lepidoptera were

associated with forms now extinct, and that only those who could change their food habits have survived.　So, as one might expect, in the forms ancestral to the Papilionidae we have a large percentage of types rather unspecialized in their food.

Behind the Papilionidae, the most plausible genealogy shows the following succession of types, representing each successive hypothetical stage of development by the modern family which most nearly shows its essential features: Hesperiidae, Euschemonidae, Castniidae, Cossidae, Tineidae, Eudarcia group, Incurvariidae, Eriocraniidae and Micropterygidae.　The following summary will show the steps that specialization took and give a hint of the time needed for the development.

MICROPTERYGIDAE.　Mouth-parts mandibulate, without sucking tongue, adapted for feeding on pollen (microspores).　Digestive system adapted for handling large amounts of protein.　Wings alike in form and venation (nearly), no specialized frenulum; the membrane spinulated (aculeate).　Female reproductive system with a single outlet, used for both vagina and ovipositor, the abdomen with ten normal segments and fleshy ovipositor adapted for laying eggs externally.　Larva feeding externally on wet moss, very fragile, with a special arrangement of setae, long antennae and small but definite compound eyes.　Pupa free and active though not feeding, with very large mandibles.

ERIOCRANIIDAE.　Mouth and digestive system completely reconstructed, without functional mandibles, but with a spiral sucking tongue, for nectar; not adapted for any solid food, the nourishment except for water and a little sugar and perhaps salt, being accumulated in the larval stage.　Reproductive system completely reconstructed for laying soft, slender eggs in the tissues of the food-plant; provided with a bladelike ovipositor with complicated muscles and tendons, the tenth segment involved in this and not recognizable as such.　Larva (side-specialized) with normal short antennae and separate ommatidia.

INCURVARIIDAE.　Wings (mainly hind wings) completely reconstructed; with a highly specialized frenulum, in the male of fused bristles, working in a hook, and with four veins of the wing lost by fusion or atrophy.　Larva with habits not significantly changed, but with the setae rearranged in the modern pattern.　Pupa still pretty active, but no longer with obvious mandibles, no longer traveling away from the cocoon.

TINEIDAE (*Eudarcia* group).[3]　Slight simplication at base of wings.　Reproductive system completely reconstructed, with complete loss of ovi-

[3] Somewhere near this point the Tischeriidae came off.　They show a transitional type of ovipositor, but are too much specialized in other ways for full interpretation.　The Opostegidae may also be a transitional type.

positor and tenth segment, vagina developed in segment ahead of ovipositor and complex internal structures developed. Egg again chitinized and laid externally. Larva becomes an external feeder, but still protected (by a case).

TINEIDAE (*Scardia-Tinea* group). *Aculeae* much reduced (*Achanodes*) and then lost (*Tinea*).[4]

COSSIDAE. Maxillary palpi become obsolete. Minor changes in venation and larval setae. Larva again a borer. In fore wing vein R_{4+5} tends to split back on R_{2+3} before origin of R_1

CASTINIIDAE. Antennae become clubbed; appearance becomes butterfly-like. R_{4+5} and upper branch of M disappear within the family.

Euschemonidae. A *butterfly*. Veins R_{4+5} M and 1st A permanently lost; R_{4+5} (as seen in pupa) consistently arises before R_1. Caterpillar again an external feeder (in a shelter) with dense secondary hair, etc. (Pupa ?).

Hesperiidae. Frenulum lost. Pupa becomes obtect (*i.e.*, nearly soldered together, with at most a little motion at three joints) within the family.

Papilionidae. Sharp tip of antenna lost; eyes enlarged at expense of middle of head; fore wings get two veins stalked; hind tibia loses a pair of spurs. Larva becomes an unprotected type, with a new kind of prolegs; pupa is hung up unprotected.

All this series of changes must have occurred in the Lepidoptera before the Jurassic, if our conclusion from the food of the Papilios is legitimate. A long time must certainly be involved, especially for the complete reconstruction of the reproductive system (twice) and the digestive system.

There is another interesting approach. We may try to synthesize the original Lepidoptera by combining the primitive features of the four or five earliest families surviving; taking the digestive and reproductive systems of *Micropteryx,* the larval body of *Hepialus* with the head of *Micropteryx,* the venation of *Micropteryx* with some extra veins in the anal region from *Eriocrania,* the legs of *Incurvaria,* etc. Putting these together we seem to have a moth of substantial size (larger than *Micropteryx* or *Eriocrania*) with a larva feeding on abundant succulent material, such as the inside of a starchy or fleshy stem, and well adapted to a very moist if not actually liquid environment; and an adult which fed

[4] The known larvae are fungus feeders, scavengers, etc., but this is certainly a side-specialization as the related forms, such as Gracilariidae, Tischeriidae, Lyonetiidae, are feeders on living plants.

freely on some abundant supply of pollen, and may have supplemented this protein diet with water or nectar.

If we take the surviving forms we find that each in a different way has adapted itself to a much less abundant or easily available nourishment. *Micropteryx* and *Eriocrania* have continued to take a succulent food, but have been reduced in size, and adapted to special conditions, *Micropteryx* living on wet moss and *Eriocrania* going through its life history in a few weeks of the spring in the tissue of newly expanding leaves, then sleeping in the soil for 11 months till this ephemeral supply is available again. The Hepialidae have remained borers, but have either taken to the storage roots in the soil (like *H. humuli*) or have adapted themselves to getting nourishment out of hard modern woods (like *Sthenopis*). The Tineidae have gone in for fungi and so on. In the adult stage, the Micropterygidae still feed on pollen, but they are reduced to a size where the small amount supplied by modern flowers is sufficient, while the others live their adult life on the proteid taken in as a larva, and either do not feed at all in the adult (Hepialidae) or have also become minute and get along with a minimum amount of nectar. It would appear, then, that the original Lepidopter was evolved in an environment with plenty of food for larva and adult, in particular with plenty of nourishing microspores for the adult proteid supply; and then went through a period when even the nectar supply of the present day was not available, but the adult had either to get on with a minute amount of nectar or go thirsty. For the great mass of modern butterflies and many moths need far more nectar than the Eriocraniidae, to keep them going.

There appears to have been one time in geological history which fills the bill, namely, the Carboniferous, followed by the very dry Triassic period needed to cause the specializations of the surviving primitive families. The Carboniferous fills it in every way; for there were

plenty of succulent stems to supply larval food and an indefinite supply of microspores for adult food. (Certain coals are largely composed of spores.)

So far as the botanical needs are concerned one could go back a little further yet, even to the curious plants of the Devonian, but the known history of the insects themselves seems to forbid us to go farther. It is only in the middle Carboniferous that we have a fossil history of the insects, and the most modern known of these belongs to the Neuroptera. Even allowing for the fact that our geological record of a form so fragile as an insect probably only begins when it becomes abundant, it would still be too daring to carry the origin of the Lepidoptera back of the known history of such things as the Palaeodictyoptera and Protoblattoids.

It is interesting to note that it is in just this period (Upper Carboniferous), which seems the most probable for the origin of the Lepidoptera, that we first come across *flowers;* that is plant reproductive organs suitable for the attraction and utilization of insects. I may take *Lyginopteris* as an example of this. It is monoecious or dioecious, and so, obviously intended for cross-fertilization. The male inflorescence is on the tips of the twigs, in a conspicuous position easily available to an insect seeking pollen for food; and the female inflorescence is surrounded with an involucre which may perhaps have been colored, and, like the rest of the plant, is well provided with glands, such as could have secreted nectar. We can imagine the moth, first taking a full meal of pollen at the male flowers, and then going to the female flower, quenching its thirst at the glands there and incidentally fertilizing the flower.[5]

[5] I have chosen Lyginopteris as a known plant from the Carboniferous, with floral structures of a type suitable for insect fertilization. There is an increasing tendency among botanists to think the Angiosperms themselves are as old as the Permian or Carboniferous, for reasons parallel to those from which I am arguing the age of the Lepidoptera. See Seward, ''Plant Life Through the Ages.''

From this time on we have a continuous succession of plants with flowers, marked by conspicuous involucres, and obviously designed for insect fertilization.

We may perhaps question if the insects for which the flowers were first adapted belonged perhaps to some other order. We immediately think of the Hymenoptera, which were undoubtedly even older than the Lepidoptera, and are now among the principal flower-fertilizers. They are certainly a possibility, but the more primitive of the Hymenoptera are better adapted in their mouth-parts to a predacious diet; and I have in fact actually seen a saw-fly eagerly eating a lady-bird. I suspect the primarily flower-feeding Hymenoptera were a little too late on the scene, but as soon as the bees had come to supplement the saw-flies, ichneumon flies and wasps, they were certainly helping out in the task of fertilization. And at present at least some of the saw-flies themselves are also among the pollen feeders, though not specialized much for the purpose.

The final possibility is that the insects that first caused the appearance of flowers were of a type now wholly extinct. This is not impossible, but there is only one type which I should consider seriously at present as a possibility—*Eugereon*. This was a strong flyer with a beak, undoubtedly; but I do not see how any insect with such mouth-parts could have made the change from pollen to nectar.

THE CORTICAL FUSI OF MAMMALIAN HAIR SHAFTS

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AMONG the microscopic structural elements of the mammalian hair shaft (Fig. 1) are minute vesicles or chambers lying among the cells of the cortex of the shaft, well known to students of trichology; but little studied; and which have been referred to under a variety of names, such as air spaces, air vacuoles, air chambers, air vesicles, air cavities; or simply as spaces, vacuoles, vesicles or chambers, as the case might be. A study of these features of hair-shaft structure was recently made by the writer, from a collection of hair samples representing all the races of mankind, kindly given to the writer by Dr. Aleš Hrdlička, of the United States National Museum, and from a collection made and sent by friends from abroad, and by Miss Elizabeth Wynkoop, of this college, during her study of the age correlation of certain features of hair-shaft structures.[1] The infrahomonid hair samples were those used by the writer in previous studies of hair-shaft structure among those mammals.[2] The comparison of the cortical air chambers or vesicles in so many different sorts of hair shafts brought to light some interesting facts regarding their origin, structure and relationships.

In the human head hair, as the hair shaft is pushed away from its papilla in the base of the follicle (the seat of the hair germinating cells), the cortex cells are not at

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[1] E. M. Wynkoop, ''A Study of the Age Correlations of the Cuticular Scales, Medullas and Shaft Diameters of Human Head Hair,'' *Amer. Jour. Phys. Anthrop.*, 13, 2, 177, July–September, 1929.

[2] L. A. Hausman, ''Structural Characteristics of the Hair of Mammals,'' AMER. NAT., 44, 496, November–December, 1920; ''Further Studies in the Relationships of the Structural Characteristics of Mammalian Hair,'' AMER. NAT., 58, 544, November–December, 1924; and ''Recent Studies in Hair Structure Relationships,'' *Sci. Mon.*, 30, 258, March, 1930.
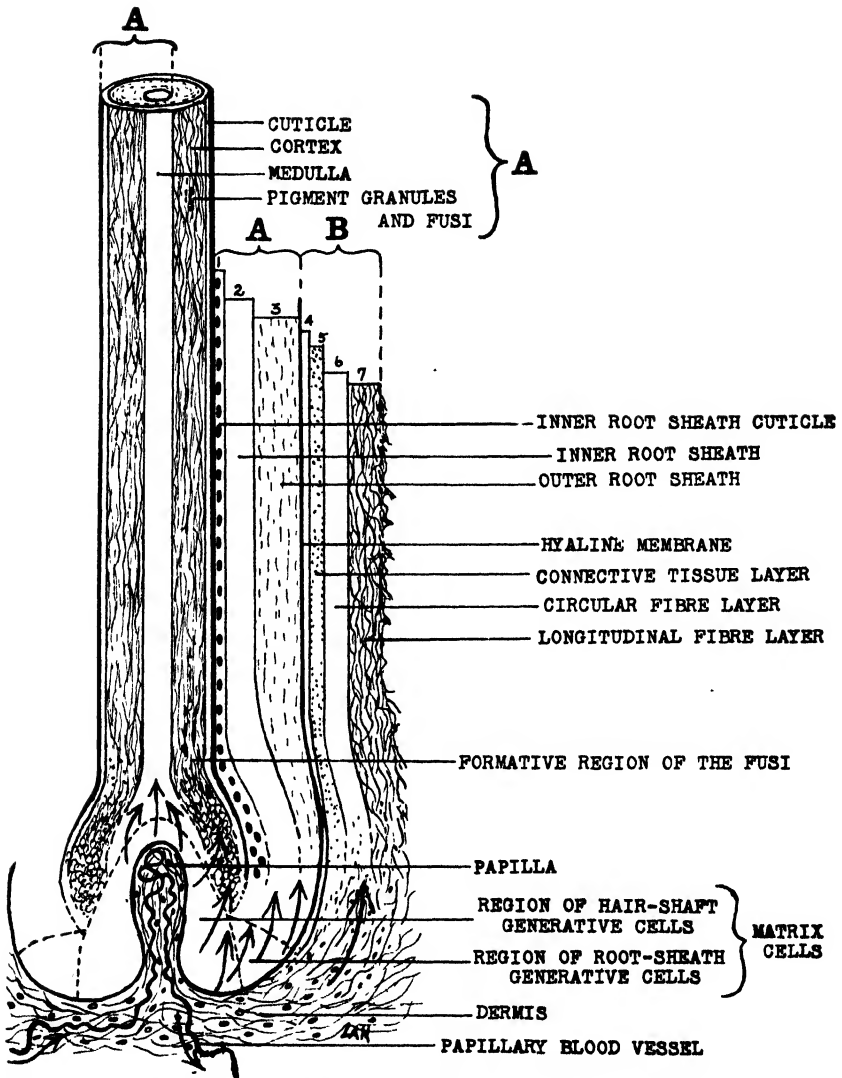
FIG. 1. Diagrammatic longitudinal section through a human head-hair shaft and its tissues of origin and support, to show the relationships of the component elements of the shaft and their generative cell layers. A, epidermal derivatives; B, dermal derivatives.

once long and fusiform, as in the mature hair shaft, but are irregularly ovoid or ellipsoid, elongating as the shaft rises toward the follicle mouth, and carrying thus upward between many of them irregularly shaped cavities filled with tissue fluid. Where these are most distinct is just above the neck of the developing hair, in a region termed the formative region of the *fusi*[3] (Figs. 1 and 2). In a hair shaft treated with potassium hydroxide, as described at the end of this paper, the fusi, just below the level of the follicle, may be studied in their relations with their surrounding cortical cells, though both cells and fusi are by this treatment slightly distorted (Fig. 3). The fusi lie between, and not within the cortical cells, though sometimes a cell may be found which appears to be hollow within, as though its nucleus had disintegrated, leaving a chamber in the center of the cell, with a few dispersed granules.

As the fusi are borne upward with the elongation of the hair shaft they become increasingly elongate, and thinner, being pressed into this shape by the drying out of the cortex. With this process also comes a loss of their tissue fluid. This occurs in the region of, or just below, the level of the mouth of the follicle (varying in different hairs). When the fusi are thus emptied of their contents they become the so-called air chambers, and become then, easier to detect among the cortical cells and their elongate nuclei, since they appear darker by transmitted light (or at least with darker heavier borders). When still filled with tissue fluid (with a density more nearly like that of the soft cytoplasmic cell substance) they are much less discernible. At the level of the mouth of the follicle, or just above or below it, they appear as fusiform chambers, varying in this general contour from short and ellipsoidal, to long and slender

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[3] Since these vesicles are roughly fusiform; and since they are characteristic of the cortex; and since they have been alluded to under so many different names, it is suggested, for the sake of simplicity and accuracy of nomenclature, that they be called the *cortical fusi,* or simply the *fusi*.
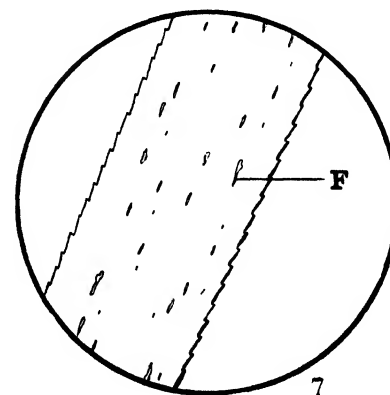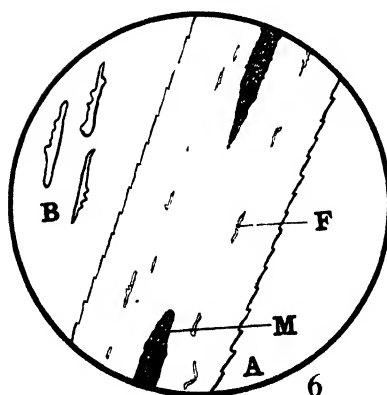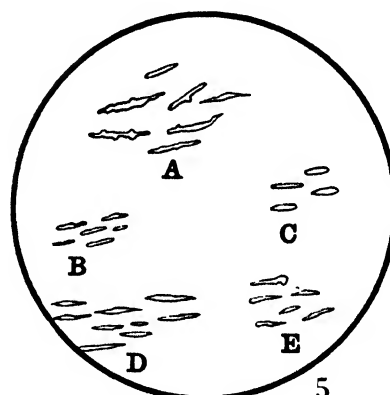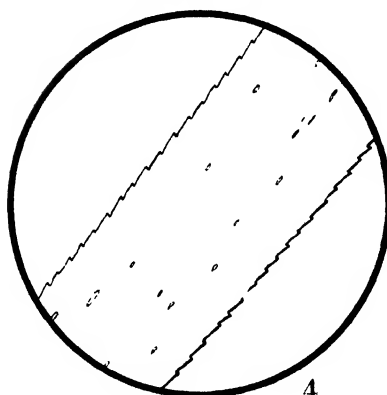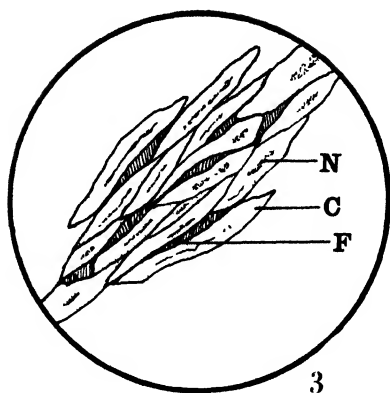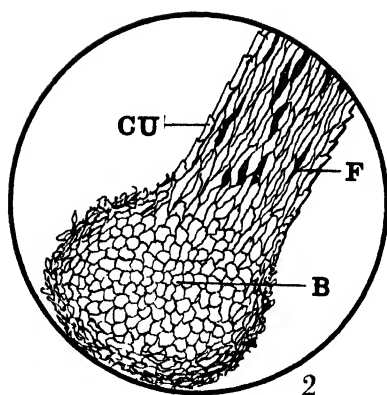
(Figs. 4 and 5). Fig. 6 shows the curious type of fusi met with in a specimen of *ringed* human head hair, an unusual condition of the hair shaft, which gives to the hair a banded appearance, light and dark zones alternating in each shaft. About forty cases only of this ringed hair were on record[4] in 1925.

Fusi vary with the region of the hair shaft. Thus, in most hairs where they are numerous at the base, they pinch out and either disappear altogether, or become extremely thin and filiform before the tip of the hair is reached. Such a condition is shown in Figs. 7, 8 and 9, in a light yellow-brown head hair. In many instances the disappearance of the fusi takes place in the distal third or quarter of the shaft, while in others fusi may persist out almost to the very tip of the shaft (Fig. 10). This may occur in white or very light yellowish shafts. In this region of the hair shaft the fusi are difficult to separate from the long striated appearances which mark the boundaries of the cortical cells. In one instance, in female head hair of a light yellow, almost white, the fusi

FIG. 2. Bulb of a hair shaft, showing the developing cortical cells and the developing fusi among them. B, bulb; CU, cuticular scales; F, fusi, lying among the cortical cells. All this below the level of the mouth of the follicle.

FIG. 3. Cells from the cortex of a head hair, just below the level of the mouth of the follicle, treated with potassium hydroxide. C, cortical cells; F, fusi; N, cortical cell nuclei. The cells and fusi are flattened and distorted; the cuticular scales have been removed.

FIG. 4. Fusi in the base of a brown hair, at the level of the follicle mouth. the medulla and pigment granules are not shown.

FIG. 5. Various types of fusi found in human hair shafts near the level of the follicle mouth. A, from the hair of the forearm; B, from light yellowish head hair; C, from brown head hair; D, from white head hair; E, from yellow-brown hair.

FIQ. 6. Fusi in a *ringed* human head hair. A, hair shaft with the fusi in situ; B, typical fusi enlarged. From the region of the mouth of the follicle.

FIG. 7. Fusi in the base of a light yellowish-brown head hair. F, fusi.

[4] C. H. Danforth, ''Hair, with Especial Reference to Hypertrichosis,'' Chicago, 1925. The sample in the writer's collection was kindly sent to him by Miss Eleanor McMullen, of Cornell University.

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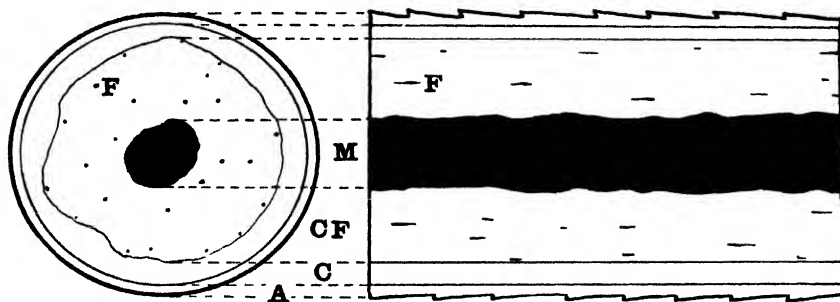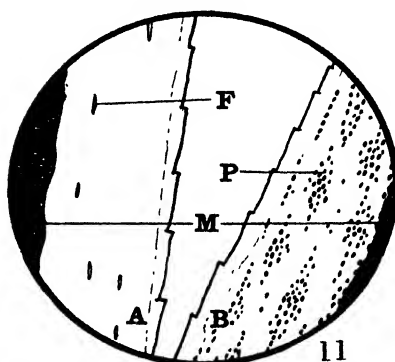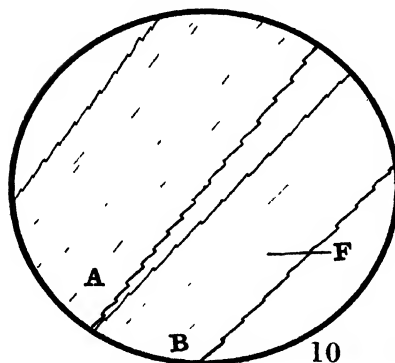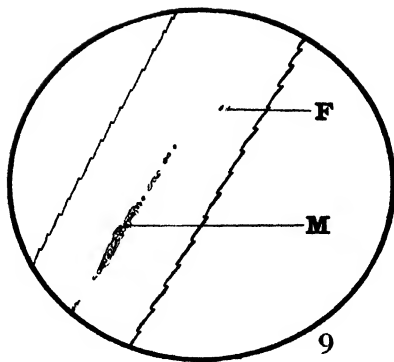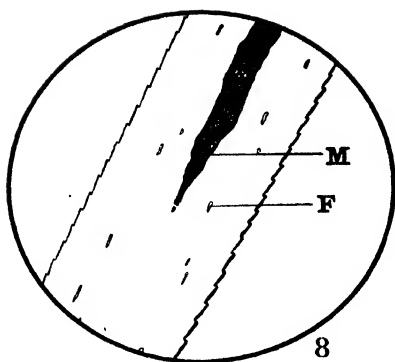
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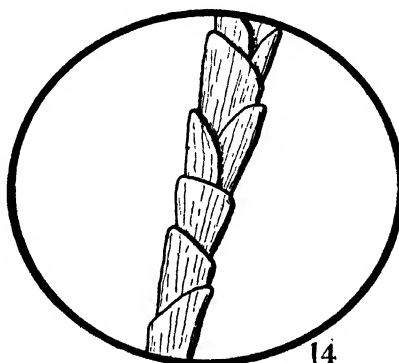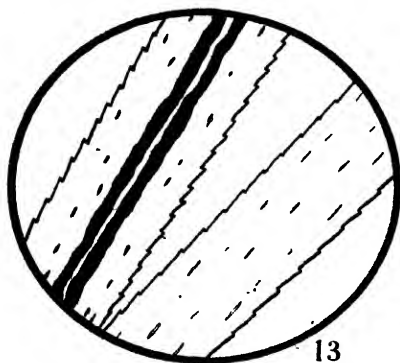
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were discernible entirely to the tips of many of the shafts. These were the natural tips, not the cut ones. This is regarded as unusual, and, in this case, when taken together with the arrangement of the few pigment granules in the cortex might have been used as a criterion for individual identification of the source of the sample. The case, also, of the Fuegian hair (to be mentioned in connection with fusi and color associations) is another instance where association of structural features might be used as definitive criteria. But such matters would require very careful study before affairs of moment could be intrusted to them as arbiters. An unusual series of samples taken from the head of an English woman at the ages of three, ten, twenty and fifty-six showed, in the yellowish hair of three, elongate fusi; in the yellowish-brown hair of ten, the same numbers but shorter; in the slightly darker hair of twenty, the same condition; and in the dark brown hair of 56 no fusi at all. All these

FIG. 8.   Fusi midway in the shaft of the same hair. F, fusi; M, medulla.

FIG. 9.   Fusi near the tip of the same hair. F, fusi; M, medulla.

FIG. 10.   A, fusi in the distal quarter of a light straw-yellow hair from one of the ''White Indians'' of San Blas. B, fusi in the tip of the shaft of the same hair. (Sample sent by Dr. Aleš Hrdlička.)

FIG. 11.   A, portion of shaft of hair from a Fuegian, showing large lenticular fusi. Pigment granules, as in shaft B, not shown. B, portion of shaft of hair of a Fingo Negro (Bantu Stock). F, fusi; P, pigment granules; M, medulla.

FIG. 12.   Diagrammatic transverse and optical longitudinal section of a shaft of head hair, to show the usual distribution of the fusi. M, medulla; A, cuticle; C, zone of the cortex usually free from fusi; CF, zone of the cortex in which the fusi usually occur when present in the shaft; F, fusi.

FIG. 13.   A, portion of the shaft of a dorsal hair from the hyena (*Hyaena hyaena bergeri*) midway between its base and tip, where it was a pure glistening white. Large fusi present in the cortex, but no pigment granules. By transmitted light the pigmentless medulla appeared nearly black. B, median portion of hair shaft of poodle, pure white, no medulla present. Fusi large and elongate.

FIG. 14.   Hair shaft of New York weasel (*Putorius noveboracensis*) just above the mouth of the follicle, showing longitudinal groovings in the cuticular scales. Shaft pure white. No fusi present.

determinations were made in the basal quarters of the shafts.

This brings us to the consideration of the association between the fusi and head hair coloration. In general, the darker the hair the fewer the fusi; the lighter the hair the more numerous the fusi. However, this admits of some interesting exceptions, exceptions which may prove useful to the microscopist who is comparing known with unknown samples. Thus, while black hair shafts, as a rule, possess no fusi, yet in the black hair of Fuegian, large, characteristically-shaped fusi occurred near the base of the shafts, clearly discernible from the thickly disposed pigment granules, and requiring only clearing and mounting of the hair shaft for examination (Fig. 11). In a hair shaft of the same color from a Negro (Fingo Tribe, Bantu Stock) with the same color and type of pigment granules and pattern, no fusi whatever could be found.

In some hairs where rough treatment, or more probably natural fragility of the shafts causes a splitting or fracturing, there occur what may be called "artificial fusi." These were found sometimes quite common in head hairs, and where they occur, may perhaps be an individual peculiarity. They seemed to be produced by a separation of the cortical cells from one another, or rarely, by the splitting of cells along the line of their elongate nuclei. They were seen only in the distal halves of shafts. Such fusi were quite large, and yet gave to the hair shaft when seen by the unaided eye an appearance no different from that of normal hairs.

The distribution of the fusi within the cortex ring is noteworthy in that it occurs some distance away from the boundary between cortex and cuticle, usually in a fairly definite zone (Fig. 12). The fusi, however, are not arranged, as far as I have observed, in a consistent chain-like fashion so common with pigment granules, nor are they grouped, like the granules, into patterns.

Among the infrahomonid mammals the distribution of the fusi varies considerably. In some white hairs the fusi are numerous, as, for example, in the pure glistening white portion of the shafts from the Hyena (*Hyaena hyaena bergeri*), and in the shafts of a sample of pure white dorsal hair from a poodle (Fig. 13). In other white hairs no fusi could be found, as, for example, in the basal portion of the pure white hair of the New York weasel (*Putorius noveboracensis*). In this basal portion (just above the level of the mouth of the follicle) minute striations appeared, which, under higher magnification, were seen to be slender longitudinal groovings in the cuticular scales (Fig. 14). In many of the light yellowish and yellowish-brown infrahomonid hairs no fusi were present, as, for example, in the light brown hairs of the intermediate bat (*Mormoops intermedia*) nor in hairs of the same hue from the proboscis monkey (*Nasalis larvatus*). In the light brown dorsal hairs of the aye-aye (*Daubentonia madagascariensis*) there occurred, near the tips of the shafts, isolated fragments of the shrunken medullary chambers, lying out in what appeared to be the cortex ring. These, at first, were mistaken for large irregular fusi. This condition was found, also, in other species, though not at all commonly. Among the mammals below man the fusi were not correlated, as they are in human head-hairs, with the coloration of the shaft, since white hairs often lacked them entirely, and darker shafts possessed them in large numbers. Whether this is a specific distinction, or an expression of some condition of the hair shaft germinating cells in the follicle only, remains to be studied.

Fusi may frequently be mistaken for pigment granules, for, when they are very minute they appear (under direct transmitted light) like solid dark bodies. But fusi and pigment granules may be differentiated by boiling the hair shaft for a few seconds in concentrated sulphuric acid; mounting it in water; and pressing it out under a cover-glass. By this treatment the fusi will be squeezed

out and only the pigment granules remain, when their shapes and sizes and relative abundance may be noted. The boiling of the shaft may be most readily done in a large drop of acid on a thick microscope slide (or in a small watch crystal) laid on the soapstone of an electric plate. Rotating the mounted shaft under a cover-glass dissociates the structural elements; floats away the cuticular scales; and disperses the long fusiform cells of the cortex. Instead of concentrated sulphuric acid one may use a strong solution of sodium or potassium hydroxide, and, after heating, mount the shaft in the same reagent, cold, or in water, as before. Large fusi may be easily distinguished from pigment granules by the clear appearance of their centers (with heavy black borders, like elongate air bubbles), by transmitted light. By reflected light they appear silvery, as do air-filled medullary chambers. To study fusi for the sake of characteristic appearance under different lights it was found best to use pure white head-hair, as here the bother of the pigment granules was removed, and one could deal with undoubted fusi. Fusi not resolvable by direct transmitted light may often be brought out by decentering the diaphragm condenser and illuminating the shaft of the hair by oblique light, in any angle desired. In the study of fusi the best results were obtained by using an objective of numerical aperture 1.30 (2 mm), with the 20 × ocular and 170 mm tube length, and an almost point illuminant, with the condenser diaphragm stopped down as far as possible consistent with sufficient illumination. Objective and condenser were both immersed with cedar oil, and a 0.17 mm cover-glass used (for which thickness the writer's objectives were corrected). For rapid determination of the presence of fusi in hair shafts, the 15 × ocular and the 3 mm objective was the combination found most useful.

SHORTER ARTICLES AND DISCUSSION

SOMATIC CHROMOSOMES OF CERTAIN MINNESOTA ORCHIDS

FROM the standpoint of the systematic botanist, a cytological study of the species in groups of plants is of value in determining phylogenetic relationships. There are between 14,000 and 16,000 species of orchids, and of this number the chromosomes of only 33 species have been studied, of which only six are native to the United States.

Most of the work on the Orchidaceae was done over 20 years ago. In 1924 Belling reported the chromosomes of *Cypripedium acaule* Ait. to be n = 10. Friemann, in 1910, reported n = 12 for *Epipactis palustris*. Brown in 1909 reported *Habenaria ciliaris* (L.) R. Bn. as having n = 16. Pace in 1909 reported *Calopogon pulchellus* (Sw.) R. Bn. as having n = 13; and in 1907 he had reported n = 11 for *Cypripedium pubescens* (Willd.) Knight, *C. spectabile* Salisb., and *C. parviflorum* Salisb. Species from other parts of the world have numbers that vary considerably, even within genera.

During the spring of 1931 root-tip material was gathered from 14 species of orchids during May and June. The tips were cut from the plants as soon as they were removed from the ground, killed in Allen's modification of Bouin's killing fluid, and stored in this solution until October, when the material was imbedded. Sections were cut 10 microns thick and stained with iodine-gentian violet. The drawings were made with the aid of a Bausch and Lomb camera lucida at a magnification of 2500.

Material from only eight of the fourteen species collected was found to have satisfactory figures. Table I lists these species and their diploid chromosome numbers. It will be noted that the three *Cypripediums*, *C. pubescens*, *C. acaule* and *C. candidum*, had 20 chromosomes in each case. *Calypso bulbosa* had 32, and the two species of *Habenaria* and the two of *Orchis* each had 42. These numbers were obtained by many counts, and were carefully verified with camera-lucida drawings.

An examination of the plate will reveal the following information. The chromosomes are very large in the species of *Cypripedium*, and are very similar in size and shape, but in *C. can-*

TABLE I

Species	Diploid chromosome number
Cypripedium acaule Ait.	20
C. candidum Muhl	20
C. pubescens (Willd.) Knight	20
Calypso bulbosa (L.) Oakes	32
Habenaria bracteata (Willd.) R. Br.	42
H. orbiculata (Pursh.) Torr.	42
Orchis spectabilis (L.)	42
Orchis rotundifolia Banks	42

didum they were somewhat longer and more slender. The range in size in the Cypripediums was from 5 to 10 or 12 microns in length by ½ to 1½ microns in diameter. In *Calypso bulbosa* the chromosomes are small, from 1½ to 2½ microns in length by less than a micron in diameter. Those of *Orchis spectabilis* and *Orchis rotundifolia* are very similar in size to those of *Calypso bulbosa* but are more numerous. There is a striking difference in size between the chromosomes of *Habenaria bracteata* and those of *H. orbiculata*. In *H. bracteata* there was one chromosome, larger than the rest, present in every complement examined.

This study has yielded several interesting facts aside from the chromosome numbers of the species. The extreme size and volume of the *Cypripedium* chromosomes are striking features. Another striking feature is the difference in size between these chromosomes and those of the other genera. There is also a marked difference in size between the chromosomes of the two species of Habenaria. The numbers for the species, so far as studied, are constant within genera.

There are some discrepancies between the chromosome numbers reported by other investigators and the numbers reported in this paper. *Habenaria ciliaris* was reported as having $n = 16$, while the two species of *Habenaria* reported here had $n = 21$. Pace reported $n = 11$ for *Cypripedium pubescens, C. spectabile* and *C. parviflorum*, while this study showed $n = 10$ for three species of *Cypripedium*, including *C. pubescens*.

I am indebted to Dr. C. O. Rosendahl, of the University of Minnesota, for help in collecting the material.

<div style="text-align:right">L. M. HUMPHREY</div>

IOWA STATE COLLEGE
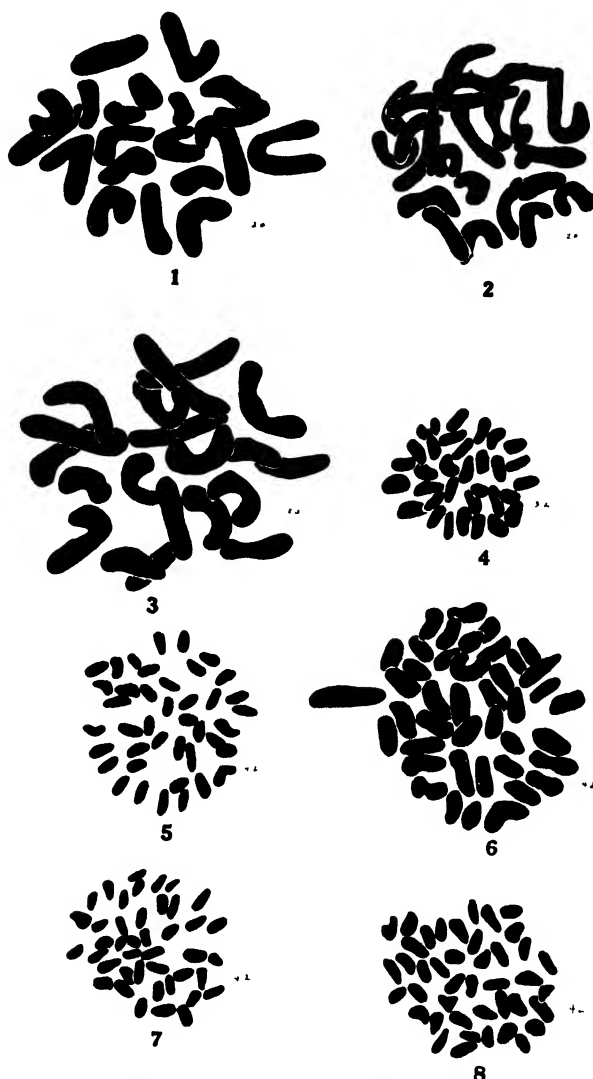
EXPLANATION OF THE PLATE

All the figures are magnified 1700 diameters

FIG. 1. The somatic chromosomes of *Cypripedium acaule*, 20
FIG. 2. The somatic chromosomes of *Cypripedium candidum*, 20
FIG. 3. The somatic chromosomes of *Cypripedium pubescens*, 20
FIG. 4. The somatic chromosomes of *Calypso bulbosa*, 32
FIG. 5. The somatic chromosomes of *Habenaria orbiculata*, 42
FIG. 6. The somatic chromosomes of *Habenaria bracteata*, 42
FIG. 7. The somatic chromosomes of *Orchis rotundifolia*, 42
FIG. 8. The somatic chromosomes of *Orchis spectabilis*, 42

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THE VERMILION MUTANT OF *DROSOPHILA HYDEI* BREEDING IN NATURE

During early September, 1931, two or three bushels of windfall peaches were gathered and placed in a large garbage can in the garden at my home in Wooster, Ohio. The *Drosophila* population was studied, as species came and went. While there are generally representatives of several species to be found about any mass of exposed decaying fruit or vegetables in this region between the months of May and November, one species is likely to be present in much larger numbers than any others. However, the dominant species changes with the changing food conditions. The rapidly breeding *Drosophila melanogaster* was present in enormous numbers in a few days, and within ten or twelve days a brood of this species had emerged. At about this time the slower breeding and larger *Drosophila hydei* began to make up a considerable proportion of the population. By the end of the month this species was occupying the scene almost to the exclusion of all others. Among several thousand flies examined at this time all were *D. hydei*, except for 14 *D. melanogaster*, 10 *D. buskii*, 3 *D. immigrans* and 1 *D. funebris*. These five

species are by far the commonest in this vicinity about houses and gardens in the autumn.

On September 27 on examination of the population several brilliant scarlet-eyed flies of the size and general appearance of *D. hydei* were observed. It was first thought that they were probably *Drosophila mulleri,* a species reported by Sturtevant (1921) from the Southern states and very similar to *D. hydei,* but with bright, vermilion eyes. Twenty-four of these flies were collected, and on examination 22 were found to be males and 2 females, and they appeared to be *D. hydei.* The distribution as to sex indicated that the scarlet eye was a sex-linked recessive mutant. Such a mutant, vermilion, had been found and described by Clausen (1923), and stocks of this mutant have been kept in our laboratory for years. Several of these mutant males were crossed singly to virgin vermilion females from stock, and all proved to be identical or allelomorphic to true vermilion.

In the meantime, in order to record more accurately the proportion of the mutant flies in the population, pupae were collected in large numbers from the garbage can and placed in cotton stoppered bottles. The flies emerging were etherized and counted. All virgin mutant females and a number of the males were saved and mated to vermilion flies from stock. They proved to be vermilions. The table below gives the number of

Date	Vermilion males	Vermilion females	Wild type males	Wild type females
Sept. 28	19	0	217	248
Sept. 29	25	2	516	563
Sept. 30	24	2	319	378
Oct. 1	8	0	184	172
Oct. 2	9	0	114	139
Oct. 6	35	1	373	393
Totals	120	5	1,723	1,893

Total population, 3,741 flies.

wild type and mutant flies appearing. Of the 1,843 males 120, or 6.5 per cent., were vermilion. Of the 1,898 females 5, or .26 per cent., were vermilion. The distribution as to sex is what

would be expected of a sex-linked recessive breeding in the general population. Since 6.5 per cent. of the males were vermilion, then 6.5 per cent. of the X-bearing sperm would be expected to carry vermilion and 6.5 per cent. of the females would be expected to receive the vermilion-bearing X. On the basis of random mating .42 per cent. of the females in the population should be homozygous vermilion. Of the total of 1,898 females 8 ± 1.9 would be expected to be vermilion. Actually, of this total 5 vermilion females were found, a fluctuation which might well be due to chance. D/P.E. is here 1.6. These calculations are made on the basis that the proportion of vermilion-bearing and non-vermilion-bearing X chromosomes remains constant through two generations, and that the vermilion males are actually as successful in mating as the wild type males.

This indicated that the mutant had been breeding for some time in the general population and that the individuals collected did not represent the immediate offspring of a few laboratory escapes of impregnated stock vermilion females. However, it is not unlikely that the original vermilion flies came from laboratory escapes, as this mutant has been raised in large numbers in our laboratory in the last few years in connection with studies on sex-linkage and non-disjunction. The population which has just been described was living a quarter of a mile from the laboratory where stock cultures are kept.

Soon after this, the vermilion mutant was observed in four different grocery and fruit stores one mile south of the laboratory, and some of these stores one eighth of a mile apart. On October 7 three of these males were taken among about thirty *D. hydei* observed on the front windows of one of the grocery stores. At this store the mutant was so abundant that one clerk had noticed the "big black red-headed fruit flies" and commented on them. Four of these vermilion males caught in grocery stores a mile from the laboratory were tested genetically and found to be true vermilion. At a point approximately three quarters of a mile northeast of the laboratory the vermilion mutant was again observed late in November among a population of *D. hydei* breeding on windfall apples.

DISCUSSION

To the geneticist mutations appear to be the building stones of evolution. To certain writers on evolution mutations seem

to be purely and exclusively the plaything of geneticists. The student of *Drosophila* recognizes that the vast majority of the mutations with which he works are deleterious in their effects upon the organism under the given set of environmental conditions under which *Drosophila* are raised. However, this does not mean that all mutations observed in *Drosophila* would necessarily be non-adaptive or detrimental under special environmental conditions and interacting with certain biotypes. Pearl, Parker and Gonzales (1923) and Gonzales (1923) have shown that the vestigial race of *D. melanogaster* is short-lived, as compared to the long-winged wild race when raised under standard culture conditions. Yet I have repeatedly observed that when vestigial and wild type flies are kept together without food and water, the vestigial on the average survive longer than the wild type. In a special environment in which one-day periods of extreme dryness alternated with periods of humidity and abundant food supply the vestigial mutant would survive and the wild type would perish. To say that such a special environment does not actually exist in nature does not invalidate the argument. To take another example, if vestigial and such multiple mutant stocks as dumpy-black-purple-curved-plexus-speck are made up in mass cultures in a number of different culture vials with the standard banana agar medium and wild type mass cultures are made up at the same time it will be found after a period of one month or six weeks that more of the mutant cultures still contain living larvae and flies. The very viability and fertility of the wild type under this special environment has resulted in the race dying out through competition and overcrowding, while the weaker, less prolific and consequently less crowded mutant forms have survived the period on the same food supply. In a special environment, where the food supply was very limited, it is conceivable that some mutant races of *Drosophila* might actually be at an advantage. Gates (1930) records a case somewhat parallel to this where horses were placed on Sable Island, Nova Scotia. In the course of 150 years the race had undergone change in form and decrease in size. He considers that inbreeding alone could not account for this and suggests that the condition may be accounted for in part on the ground of mutations and the selective action of the environment on the smallest in size and consequently the best adapted to the limited food supply.

Vermilion is one of the hardier mutant types of *D. hydei*, and in laboratory cultures holds its own very well in competition with the wild type. In this connection an interesting observation was made by W. S. Wilde. Working at Miami University in 1930 and 1931 on the phototropic responses of *D. hydei* wild type and some of its eye-color mutants, he found that vermilion was more active and responsive than the wild type stock used.

The evidence presented above indicates that during the season of 1931 the vermilion mutant, either from laboratory escapes or from a mutation occurring in nature, was increasing in absolute numbers and possibly in relative numbers in the general population of *D. hydei* in and about Wooster. This does not imply that the mutant is more viable than the wild type. It does mean, however, that this mutant is not necessarily a weakling, which must be coddled in the culture bottle of the geneticist. It could live long enough in this locality in the season of 1931 to appear in several different situations and to show the ratio between the sexes of a well-established sex-linked recessive breeding in the general population. Were the vermilion mutant to have distinctly superior adaptive value over the entire range of *D. hydei*, one would suppose that the mutation would have occurred long since in nature and by natural selection would have replaced the wild type. It appears much more likely that it is not superior nor markedly inferior to the wild type on the average. It is just possible that in certain biotypes, interacting with the remainder of the genetic complex, it may condition characteristics, not necessarily eye color, which have a positive adaptive value under such an environment as that in and around Wooster in 1931.

<div align="right">WARREN P. SPENCER.</div>

THE COLLEGE OF WOOSTER

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NOTE ON A FALLACIOUS METHOD OF AVOIDING SELECTION

In a prolonged experiment which gives strong evidence for the Lamarckian transmission of an acquired disposition in rats, Mc-Dougall ('27, '30) describes the obviously very conscientious methods used to avoid selection. The selection of animals for training was generally made at random. However, to quote Mc-Dougall's works ('27, p. 274) ''In a few cases in which this random selection of young animals was not effected before training began, we chose for breeding the most and least successful member of each litter trained.'' As this procedure was only seldom adopted it can not, I think, account for McDougall's results, but it should certainly not be copied in future experiments, as it tends to pick out recessive genes.

Suppose the nth generation of a random mating population to contain a dominant and recessive gene pair in the ratios $q_nA : p_na$, where $p_n + q_n = 1$. The three genotypes will be in the ratios $q_n^2AA : 2p_nq_nAa : p_n^2aa$. The six types of family will occur in the following proportions:

All AA	q_n^4
1 AA : 1 Aa	$4p_nq_n^3$
All Aa	$2p_n^2q_n^2$
1 AA : 2 Aa : 1 aa	$4p_n^2q_n^2$
1 Aa : 1 aa	$4p_n^3q_n$
All aa	p_n^4

Selection of any kind will only operate on families of the fourth and fifth types. On the fifth type the process considered will operate impartially. For in large families we shall always pick out one dominant (Aa) and one recessive (aa). But on the fourth type it will not be impartial. There will be a strong tendency to pick out a recessive as the ''best'' or ''worst'' type.

In a large enough family a dominant and a recessive would always be selected, and in a large group of such families equal numbers of dominants and recessives would be selected, provided the observed variation were mainly due to the gene in question. In this case it can easily be shown that in the next generation

$$p_{n+1} = p_n + \tfrac{2}{3} p_n^2 q_n^2.$$

Supposing we started with $p_n = 1$, *i.e.*, $\frac{1}{4}$ recessives, the percentage of recessives in succeeding generations would be 25, 29.4, 34.0, 38.7, 43.4, 48.0, 52.3, and so on. The number of recessives would thus increase fairly quickly, and selection would be far from impartial. If families are small the increase is slower, but in the same direction. Moreover as recessive mutations are commoner than dominant, it would generally cause any mutant type which occurred to spread through the population. As the repetition of McDougall's experiment is one of the urgent tasks of geneticists, and is, I understand, already under way, it seems desirable that this possible source of error, as well as those pointed out by Sonneborn ('31), should be avoided.

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RECENT CONTRIBUTIONS TO PLANT EVOLUTION

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THE past decade has seen a marked advance in our knowledge of the early history of the vascular plants, especially the discovery in the Devonian of a number of very simple, generalized forms which seem to foreshadow the more specialized pteridophytes of the later Devonian and Carboniferous, and through these the ancestors of the modern floras.[1] These discoveries have aroused a new interest in the phylogeny of the vascular plants, and this is shown by the recent publication of two important contributions to these problems.[2]

The "vascular" plants comprise the pteridophytes—ferns, etc., and the seed-plants, "spermatophytes." These are the predominant plants of the present day. Remains of vascular plants are first encountered in the lower Devonian rocks.

There is pretty general agreement that the ancestors of the higher plants were aquatic organisms, similar to some of the algae still living; but how the first land-plants arose from their algal ancestors is a matter of much controversy.

[1] Lang, "Contributions to the Study of the Old Red Sandstone Flora of Scotland," Trans. Roy. Soc. Edinb., Vol. 54, 1925; R. Kräusel and H. Weyland. Beiträge zur Kenntnis der Devonflora. Abhand. Senck. Naturforsch. Gesellsch. 40, II. 1924.

[2] A. C. Seward, "Plant Life through the Ages." Cambridge University Press. 1931; W. Zimmermann, "Die Phylogenie der Pflanzen." Jena, 1930.

In all typical plants with sexual reproduction there is an "alternation of generations." The sexual cells, "gametes," unite to form a "zygote," whose nucleus has double the number of chromosomes found in the gametes, *i.e.*, is diploid. The complicated plants we know as the fern or flowering plant is the product of the continued growth and differentiation of the unicellular zygote resulting from the union of the male and female gametes; and the nuclei of all the cells retain the diploid character of the zygote. In order that the original "haploid" condition may be restored, a peculiar type of nuclear division is necessary, known as a "reduction" division or "meiosis."

The life-cycle of the diploid "sporophyte" is completed by the formation of special reproductive cells—spores. These are formed in tetrads from special cells—"spore mother-cells"—and the first nuclear division of the mother-cell is a reduction division, and the last division results in four spores having the original haploid chromosome number. From these haploid spores the new generation of sexual plants—the gametophytes—arises, producing the gametes from whose union the sporophyte develops.

The question as to whether the highly specialized sporophyte is a modification of the sexual gametophyte, or whether it is an independent neutral structure interpolated between the sexual generations, has aroused much controversy. The writer believes the evidence for the latter theory, *i.e.*, the so-called "antithetic" theory of the alternation of generations, is the more convincing.[3]

In the fern we see the marked difference between the very simple sexual plant and the large and complex neutral generation. From the germinating spore, there develops the minute flat liverwort-like "gametophyte" bearing the sex organs, archegonium and antheridium—this gametophyte is, in short, the sexual phase of the

[3] For a more extended discussion of the subject see the writer's "Mosses and Ferns," 3rd ed., Chap. XV.

fern's life.   The female gamete, the egg, contained in the archegonium, is fertilized by the active male gamete—
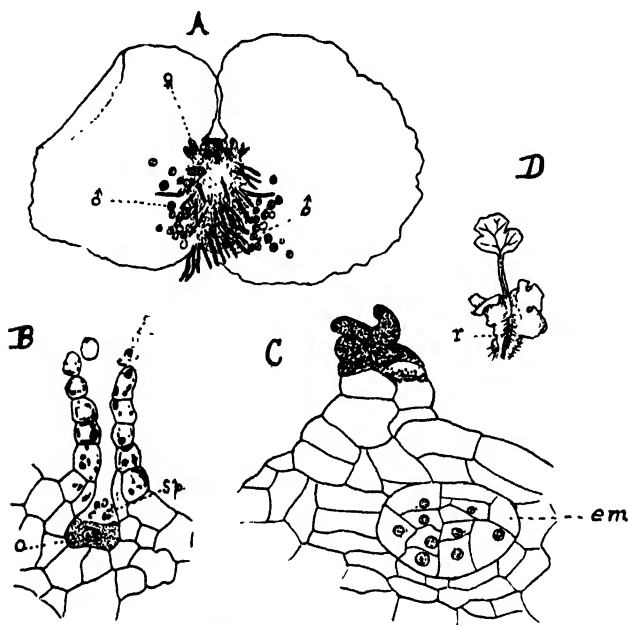
Fig. 1.   A, gametophyte of a fern, *Gleichenia*, showing male and female reproductive organs.   B, an open archegonium—o, the egg-cell, sp., spermatozoids.   C, an embryo, em.   D, the young sporophyte attached to the gametophyte, g; r, the primary root.

the spermatozoid.   The resulting zygote remains within the archegonium, where it develops into a globular or oval mass of cells, the embryo, which is protected by the surrounding tissue of the gametophyte until it has formed the primary organs of the young fern—stem, leaf and root.   By the development of the root, which penetrates the substratum, the young plant no longer is dependent on the gametophyte for its support and the gametophyte usually dies, leaving the little fern rooted in the ground. This is the "sporophyte," which sooner or later produces the spores.   As these spores are the product of simple cell-division, *i.e.*, are asexual, the sporophyte represents the non-sexual or neutral phase of the fern's life history.

In all the vascular plants the sporophyte arises in an analogous manner, and this is true also for the liverworts

and mosses where, however, the sporophyte remains permanently connected with the gametophyte, and dies as soon as the spores are shed.

Since the sporophyte of all the higher plants begins as an undifferentiated embryo, in contrast with the green algae where the zygote develops into a simple resting spore, the name "embryophyte" has been proposed to include all the plants above the algae.

Our knowledge of the evolution of the mosses and liverworts is mainly derived from a study of existing species, as the known fossil remains of these plants are very scanty.   This is due in part to the delicate tissues of most of them, but the recent discovery of unmistakable liverworts in the Carboniferous suggests that a more intensive search for these delicate plants may reveal something of their geological history.

A comparative study of the development of the sporophyte in the living bryophytes reveals some of the factors which seem to have been concerned in the establishment of the independent sporophyte of the vascular plants.

The simplest known sporophyte is that of certain liverworts of the genus Riccia.   The adult sporophyte is a globular capsule filled with spore-tetrads.   Throughout its development it is embedded in the gametophyte, from which it derives its nourishment.   In short, the sporophyte is completely parasitic.   In most liverworts, however, only a part of the embryo is devoted to spore-formation.   A special organ, the foot, anchors the young sporophyte in the tissues of the gametophyte, and also acts as a haustorium through which nourishment is supplied to the growing sporophyte.   The terminal portion becomes a spore capsule, but some of the sporogenous cells remain undivided and form peculiar elongated cells, elaters, which are concerned with the opening of the capsule and the scattering of the spores.   Between the foot and the capsule there is a stalk, "seta," which may attain a length of several centimeters in some cases.
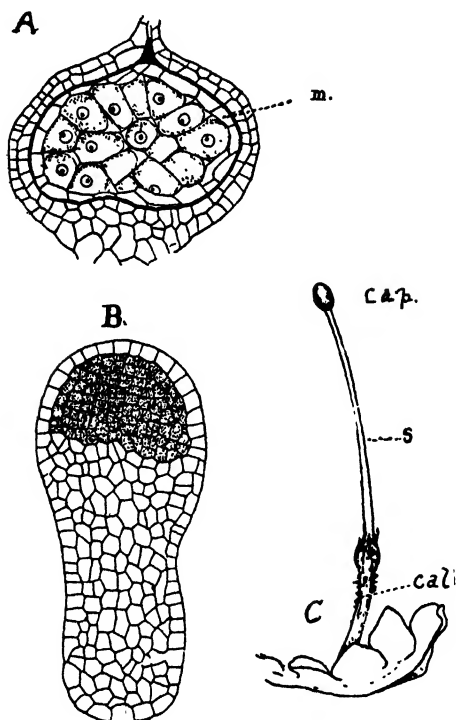
FIG. 2. A, young sporophyte of a liverwort, Riccia, enclosed in the archegonium. All the cells, except the layer, m, develop spores. B, another liverwort, Fimbriaria. The sporogenous tissue is shaded. C, ripe sporophyte of Treubia—the spore capsule borne on a long stalk, s.

The great importance of this "sterilization" of potentially sporogenous tissue in the evolution of the sporophyte has been emphasized by Professor Bower.[4] In all the liverworts the sporophyte remains intimately associated with the gametophyte, and its specialization is concerned with the formation and dissemination of the spores, the sporophytes quickly collapsing after their discharge.

In two classes of bryophytes, however, the sporophyte attains a considerable degree of independence, *viz.*, the Anthocerotes and the true mosses. In both of these the sporophyte may continue to grow for a long period, and attain a relatively large size. This growth is accompanied by a great reduction in the sporogenous tissue,

[4] F. O. Bower, "The Origin of a Land Flora." London, 1908.
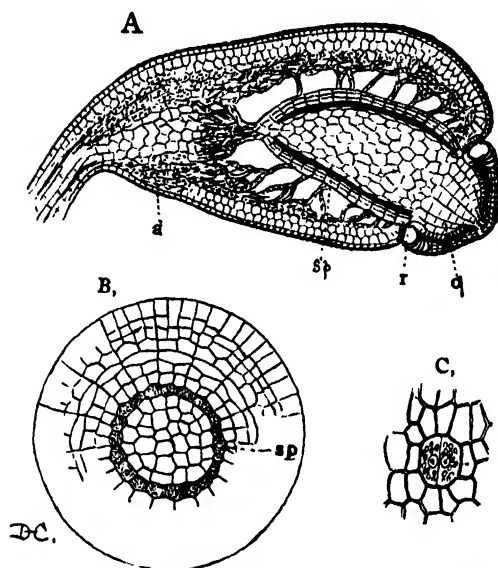
FIG. 3. A, longitudinal section of the spore-capsule of a moss, Funaria. The sporogenous tissue, sp. greatly reduced. B, cross-section of a young sporophyte, showing the single layer of sporogenous cells. C, a stoma from the base of the capsule.

and the development of a large amount of green tissue, by means of which the sporophyte can manufacture carbon compounds needed for its growth. There may be developed also a strand of conducting tissue comparable with the fibro-vascular bundles of the simplest vascular plants.

In some of the true mosses there is developed a very complicated mechanism for discharging the spores, and well-developed conducting tissue, as well as a perfect system of photosynthetic tissues. Nevertheless, there is little indication of a tendency, on the part of the highly specialized sporophyte, which in the mosses attains a complexity quite unmatched among the liverworts, and is adapted to a much greater range of conditions in the environment, to become independent.

In the Anthocerotes, although the sporophyte may reach a size comparable to that of the mosses, and like them has a marked reduction in the amount of sporogenous tissue, and a corresponding increase in the green

tissue, it is much less specialized than that of the mosses. In Anthoceros, the sporophyte is a slender cylindrical green body, with a large foot embedded in the gametophyte. There is no elaborate mechanism for distributing the spores, which may continue to form for months after the first ones are discharged, and the growth of the sporophyte is not checked by the ripening of the first spores. The continued growth is due to the development of a zone of actively dividing cells between the large foot and the upper part of the sporophyte. The foot provides the necessary water for the growth of the sporophyte, while the abundant green tissue manufactures the necessary carbon compounds, through photosynthesis. Were the
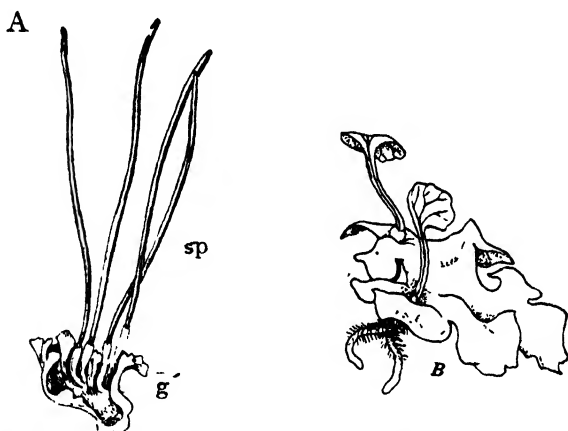
Fig. 4.   A, gametophyte of Anthoceros, bearing four sporophytes.   B, gametophyte of a fern, Danaea, with two young sporophytes.

foot able to procure water directly from the ground, the sporophyte, like that of the young fern, would no longer need the gametophyte, and indeed in some exceptional instances,[5] such actually seems to be the case. In some of these sporophytes it was significant that in the later formed basal tissue the sporogenous cells were almost wanting, and there was a marked increase in the amount of green tissue. A central core of presumably conducting cells, directly comparable to the primary vascular

[5] D. H. Campbell, ''Annals of Botany.''   July, 1924.

bundle in some of the primitive ferns, replaced the slender columella ordinarily present.

The writer has long maintained that Anthoceros, more than any other known form, suggests what may have been the structure of the first vascular plants. This view has been confirmed by the actual discovery of what, up to the present, are the simplest known true vascular plants.

About sixteen years ago there were discovered in Devonian rocks—the Old Red Sandstone—of Scotland, the petrified remains of what had evidently been a peat-bog.[6] The plant fragments were preserved with extraordinary perfection, and a study of these showed the presence of certain extremely simple plants differing so much from any known forms that a special family, Rhyniaceae, was established to contain them. The perfect petrifaction of these remains enabled a complete study, not only of their external characters, but of their anatomy and even their fructification.

The resemblance in structure between these fossils, and

A   B
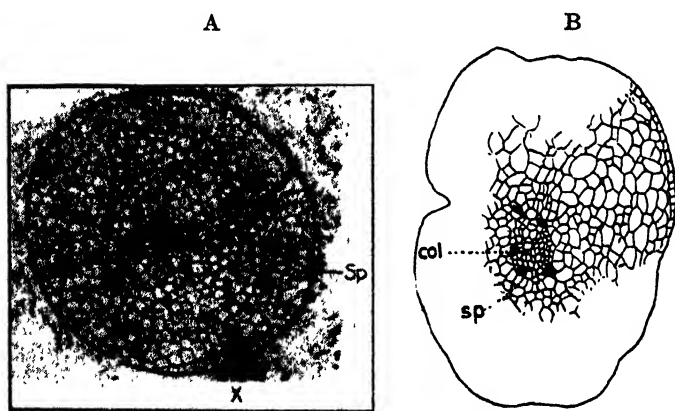
Fig. 5. A, cross-section of the shoot of Rhynia, the simplest known vascular plant, from the Devonian. B, a similar section of the sporophyte of large sporophyte of *Anthoceros fusiformis*. A, after Zimmermann.

the sporophytes of some of the existing Anthocerotes, is quite extraordinary, especially when compared with the large specimens of Anthoceros already mentioned.

6 R. Kidston and W. H. Lang, ''On Old Red Sandstone Plants Showing Structure.'' Pt. I. Rhynia. Trans. Roy. Soc. Edinb., 51, III, 1917.

The Rhyniaceae include two genera, Rhynia and Hornea. The first species described, *Rhynia Gwynne-Vaughnii,* was a slender leafless plant, sometimes dichotomously branched. The shoot arose from a prostrate rhizome, structurally much like the upright shoots. No roots were present. In size, the smaller specimens scarcely exceeded the largest Anthoceros sporophytes. Sections of the shoot of Rhynia show a central vascular bundle with a core of woody tissue, the rest of the shoot being composed of uniform thin-walled parenchyma. In the more slender shoots the woody tissue may be reduced to two or three tracheids, and in the smallest branches tracheary tissue may be quite absent. Sections of the largest Anthoceros sporophytes show a structure almost identical with that of Rhynia except for the complete absence of woody tissue.

Owing to the permanence of the woody tissues, the vascular bundles are often preserved very perfectly as fossils, and are of great importance in establishing the relationships of many fossil plants. Nevertheless, caution is necessary and it is not always safe to base relationships on woody structures alone. This may be illustrated by a living instance. The two genera, Ophioglossum and Botrychium, are placed by taxonomists in a single family, Ophioglossaceae; yet their stem anatomy is almost as diverse as that of a typical monocotyledon and dicotyledon. On the other hand, among fossils belonging to widely divergent phyla similar vascular bundles are present, *e.g.,* the development of secondary wood in many unrelated forms.

The sporophyte of Rhynia is not very much advanced beyond that of the largest known types of Anthoceros. The plant body had not yet developed the definite organs of the typical vascular plants, *viz.,* stem, leaf and root, but showed the first step in the formation of external organs by the development of dichotomous branches. Zimmermann[7] has proposed for such an undifferentiated

7 *Loc. cit.,* p. 65.

dichotomously branched plant body the term "telome," and this term is also applied to the ultimate branches of such a telome system. The branches of the telome may be either fertile or sterile.

In Rhynia spores are produced at the apex of some of the branches. The mass of spores is covered by several layers of cells, including the epidermis, and this fertile tip of the branch forms a very primitive sort of sporangium.
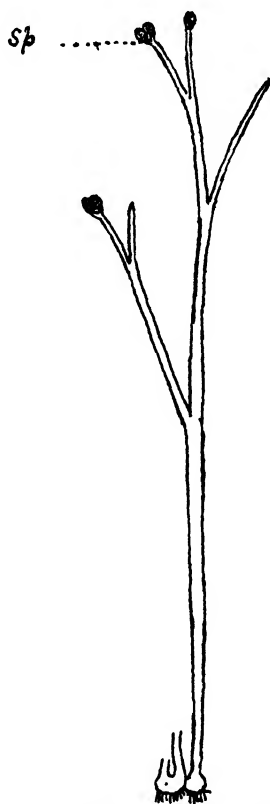
Fig. 6. *Hornea Lignieri.* Restoration after Kidston and Lang. sp. sporangia.

A second genus, Hornea, is much like Rhynia in form, but differs in some particulars. The "rhizome," instead of being elongated, and similar to the upright shoots, is a tuberous body, destitute of any vascular bundle, and strongly suggesting the large foot of the sporophyte of

Anthoceros.   An English botanist has described Hornea
"As in fact little more than a slightly ramified and free
growing Anthoceros."[8]   The resemblance to the Antho-
cerotes is increased by the origin of the sporogenous tis-
sue.   A section of the tip of a fertile branch shows that
the spores form a thick layer overarching a central mass
of sterile tissue, or columella, a condition characteristic
of all the Anthocerotaceae.   A comparison of a section
of Hornea and the simplest of the Anthocerotaceae, Noto-
thylas, where the sporogenous tissue is more abundant
than in Anthoceros, is significant.
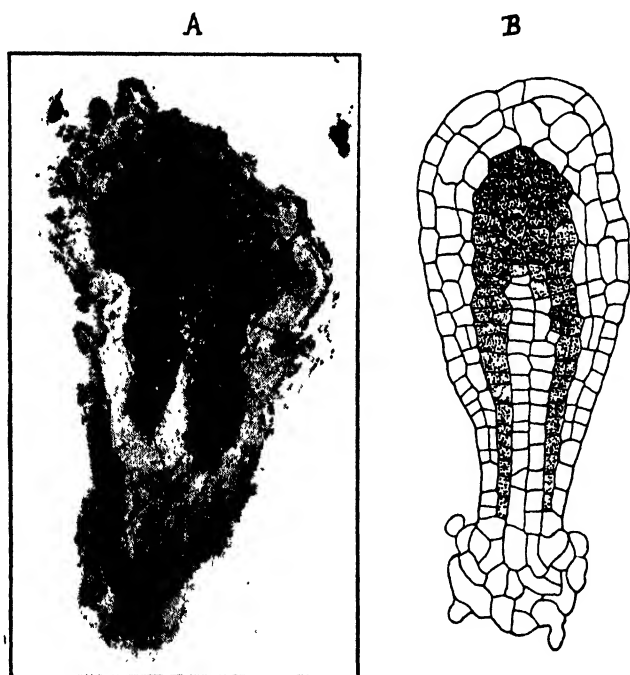
Fig. 7. Longitudinal section of a sporangium of Hornea (A), compared
with a similar section of a young sporophyte of Notothylas (B),
one of the Anthocerotes. A, after Zimmermann.

From the study of these earliest known vascular plants,
and their obvious resemblances to the Anthocerotes,
which among the existing bryophytes approach most
nearly the independent sporophyte of the pteridophytes,

[8] Seward, *loc. cit.*, p. 30.

it is justifiable to conclude that the ancestors of the first vascular plants were, if not actually Anthocerotes, at any rate were very much like them.

There is strong evidence that the Anthocerotes are very old types. The gametophyte more nearly resembles the green algae than does that of any other Archegoniate. On the other hand, the sporophyte comes nearest to complete independence of any of the bryophytes; and, except for the absence of tracheary tissue and lack of branching, can readily be compared with the undifferentiated "telome" of the Rhyniaceae. It is quite conceivable that, like the still more ancient ancestors of the higher plants, the green algae, the existing Anthocerotes are the little changed descendants of plants that flourished long before the first vascular plants appeared upon the earth.

Other important discoveries of Devonian plants of very primitive structures have been made, which seem to foreshadow the principal classes of living pteridophytes. The most remarkable forms were somewhat more advanced than the Rhyniaceae, but show evident resemblances to them. These have been described in a number of important papers by Professor R. Kräusel, of Frankfurt, and some of his collaborators. These were discovered near Elberfeld in the Rhine Valley.

One of these, Asteroxylon, apparently resembled in habit some of the living species of Lycopodium, and the structure of the vascular cylinder, or stele, was also comparable with that of Lycopodium. Kräusel has figured a restoration of Asteroxylon, which shows upright, much branched shoots arising from a prostrate rhizome, much as in the living club-mosses. Like these, also, the branching was dichotomous. The larger axes were covered with small appendages—perhaps rudimentary leaves—but the terminal shoots were smooth and coiled like the young frond of a fern.

It is quite conceivable that the Asteroxylon type might have come from a form like Rhynia by the development

FIG. 8.   Asteroxylon, a Devonian fossil, suggesting the living club-mosses. Restoration after Kidston and Lang.

of small superficial leaves, and a further development of the axial fibro-vascular cylinder.   It is also conceivable that there may be a real connection between Asteroxylon and the club-mosses (Lycopsida).

One small family of living pteridophytes, the Psilotaceae, show such resemblances to the Rhyniaceae that their inclusion in the same class, Psilophyta, is probably warranted.   The Psilotaceae comprise only a few species, one of which, *Psilotum triquetrum*, occurs in most tropical and subtropical regions.   The second genus, Tmesipteris, is restricted to the Australasian and Polynesian regions.   The upright dichotomously branched shoots of Psilotum are practically leafless and arise from
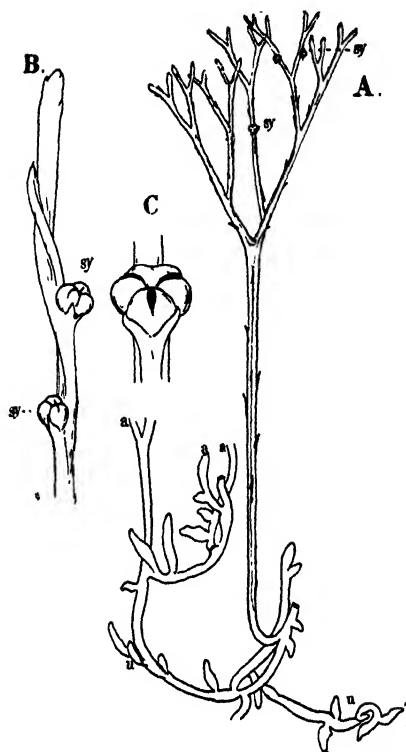
FIG. 9. *Psilotum triquetrum,* probably the nearest living relative of the Devonian Rhyniaceae. After Bertrand.

a rhizome, as in Rhynia, and like Rhynia it is destitute of true roots. The sporangia are in groups of three and are formed at the apex of short special branches. We may consider the Psilotaceae as relicts of an extremely ancient and almost extinct class.

With the fossil Psilophyta are associated many extinct types, which show more or less evident relationship with the three principal classes of living Pteridophytes, *viz.,* the club-mosses, Lycopsida; the fern-alliance, Pteropsida; and the Equisetineae (horsetails), Articulatae.

All three classes can be traced back to the Devonian, and some of the middle Devonian fossils, described by Kräusel and others, might be interpreted as synthetic types connecting the modern ones with forms like the Rhyniaceae.

The differentiation of the plant-body seems to have proceeded along two lines. In one direction the result was a dichotomously branched axis, bearing many small leaves, with a single median vascular bundle. A massive stele occupies the axis of the shoot. This type is exemplified by the living species of Lycopodium, and is characteristic of the class Lycopsida. Among the fossil lycopods are forms closely resembling the living species and probably directly related to them. But from the later Devonian and through the Carboniferous, the Lycopsida developed into trees, of which species of Lepidodendron and Sigillaria are among the most characteristic fossils of the later Paleozoic. The structure of the wood was very much like that of the living conifers, and the leaves were not unlike in structure, and it has even been suggested that these arborescent club-mosses may have been related to the existing conifers, although it must be said this view is not generally accepted.

While there is some evidence for the persistence of a few relatives of these giant club-mosses in the early Mesozoic, they were no longer a dominant feature in the floras, and their place was taken by numerous conifers, which for a long time dominated the Mesozoic forests.

There is abundant evidence that some of the fossil lycopods were heterosporous like the living genus Selaginella; but there were also forms, *e.g.,* Lepidocarpon, in which true seeds were developed, and it is this fact which has suggested that the conifers might be descended from seed-bearing lycopods. Whether or not this theory is correct, the giant lycopods of the Carboniferous became practically extinct by the end of the Paleozoic.

A characteristic of all existing lycopods is the structure of the spermatozoids, which are biciliate, in which respect they agree with the bryophytes, including Anthoceros. Whether or not the Rhyniaceae resembled the lycopods in this important character is, of course, useless to speculate. All the other living pteridophytes, Psilotaceae, ferns and horsetails have large multiciliate sperms, and

on this basis the pteridophytes have been divided into two categories,[9] Biciliatae and Polyciliatae.

We have already seen that the living Psilotaceae show evidences of a real relationship with the Devonian Rhyniaceae; but evidences of a similar relationship with the other Polyciliatae is not so clear. However, some of the recently discovered Devonian fossils may indicate the possibility of a derivation of the Articulatae and Pteropsida from Psilophyta, and also a remote relationship between the horsetails and the lower ferns—a view suggested by the writer many years ago. This conclusion was based upon marked resemblances in the gametophyte and embryo, although the contrast between the hollow-jointed stem and rudimentary leaves of Equisetum and the short solid stem and large and complex fronds of the fern would seem to make any relationship extremely unlikely. However, in the oldest-known examples of the Articulatae, *e.g.,* Asterocalamites, the leaves are much more conspicuous and are repeatedly dichotomous, very much as in many ferns.

In the Rhyniaceae, the forked plant-body (telome) shows both fertile and sterile branches, which have been denominated respectively "sporangiophore" and "phylloid." By repeated dichotomy in one plane, such a plant-body would result in a fan-shaped structure, not yet clearly differentiated into stem and leaf. A flattening of the branches would then result in a fan-shaped frond much like the leaves of many living ferns, *e.g.,* *Schizaea dichotoma,* Dipteris, Matonia.

From a study of the development of the most primitive of the living ferns, it seems probable that in the ancestors of the modern ferns the sporophyte consisted of a single large leaf and a "protocorm" or foot. The root was a later development, arising endogenously and piercing the foot, and forming a much more efficient organ for water absorption.

[9] J. P. Lotsy. Botanische Stammesgeschichte. Vol. 2: 447, 1909.
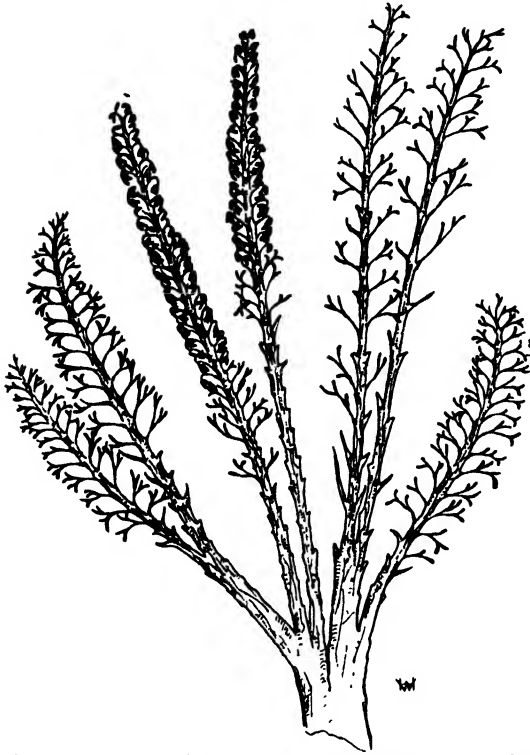
FIG. 10. Hyenia, a Devonian fossil, perhaps related to the ferns. Restoration after Kräusel and Weyland.

It is possible that such forms as the Devonian Cladoxylon and Hyenia may resemble the predecessors of the modern ferns. Hyenia, according to Kräusel's restoration of this remarkable plant, was a fan-shaped body suggesting the frond of a fern, but the branches were beset with slender dichotomously branched appendages, some of which were sporangiophores having at their apices pendant sporangia, recalling the sporangiophores of Equisetum. The branching of the main plant-body, and the absence of any indication of the jointed axis of Equisetum, is very different from the condition in any of the typical Articulatae. Much resembling Hyenia in general form is another remarkable plant, Calamophyton, also described by Kräusel. Obviously related to Hyenia, it differs in having the main branches distinctly jointed, and from the nodes arose lateral branches, much as in
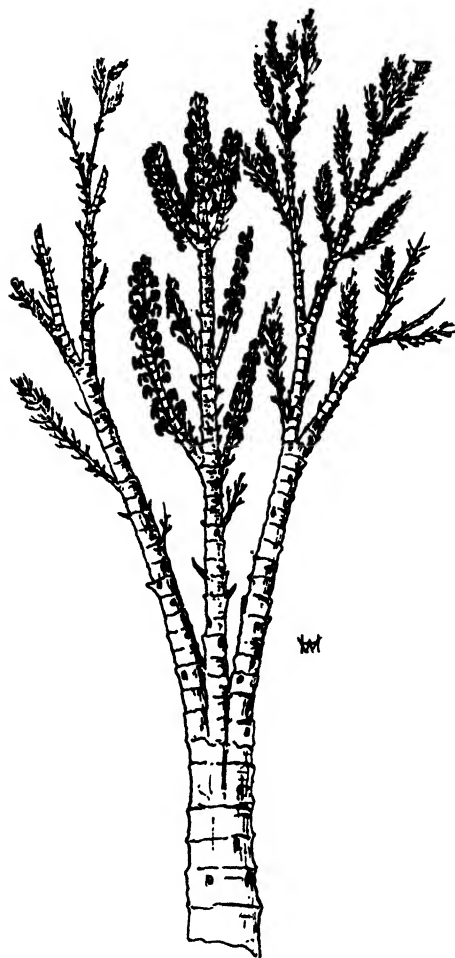
FIG. 11.   Calamophyton, a Devonian fossil, showing possible relationship
with the horsetails (Equisetineae).   Restoration after
Kräusel and Weyland.

the living Equisetum.   From the nodes of some of the
lateral shoots, whorls of small forked leaves comparable
to those of Asterocalamites, were present, while from
other nodes were produced sporangiophores much like
those of Hyenia.   In short it is quite conceivable that
from forms resembling Hyenia and Calamophyton with
their fern-like fronds, there developed in one direction
the typically megaphyllous (and primarily monophyl-
lous) ferns, and in another direction, through forms like

Hyenia and Calamophyton, the primitive Asterocalamites where the relatively large leaves retain the primitive dichotomous branching.

With a progressive reduction of the leaves, and a correspondingly increased importance of the axis, we may infer that the condition found in the sole remaining representatives of the class, the genus Equisetum has arisen.

As in the lycopods, it is probable that the living Equisetaceae are the descendants of the less specialized Paleozoic types, while the tree-like Calamites of the Carboniferous and the Sphenophyllales have left no descendants and have given way to the seed-plants. Heterospory has been demonstrated in a few of the Calamites, but no evidence of seeds in any of them has been discovered; and all that remains of the class at the present time is the single genus Equisetum, with some 25 to 30 species.

Of the living ferns there is good reason to believe that the Ophioglossaceae are the most primitive. Unfortunately, perhaps due to their delicate structure, practically nothing is known of their geological history. It is, however, not unlikely that an extinct order of ferns, characteristic of the later Devonian and lower Carboniferous, the Coenopteridales, may have been related to the Ophioglossaceae.

The young sporophyte of *Ophioglossum pedunculosum* (*O. moluccanum*) shows a structure suggestive of Rhynia or Anthoceros.

It consists at first of an apical region, which develops into the primary leaf and a foot; but very early there is formed endogenously near the base of the leaf a root which penetrates the foot and grows vertically into the substratum. The root and leaf are in the same plane, and the young sporophyte is thus distinctly bipolar. There is an axial stele which extends unbroken through root and leaf. Presumably, in the ancestral form, the apical region was sporogenous, but in the living species the development of spores does not take place until a
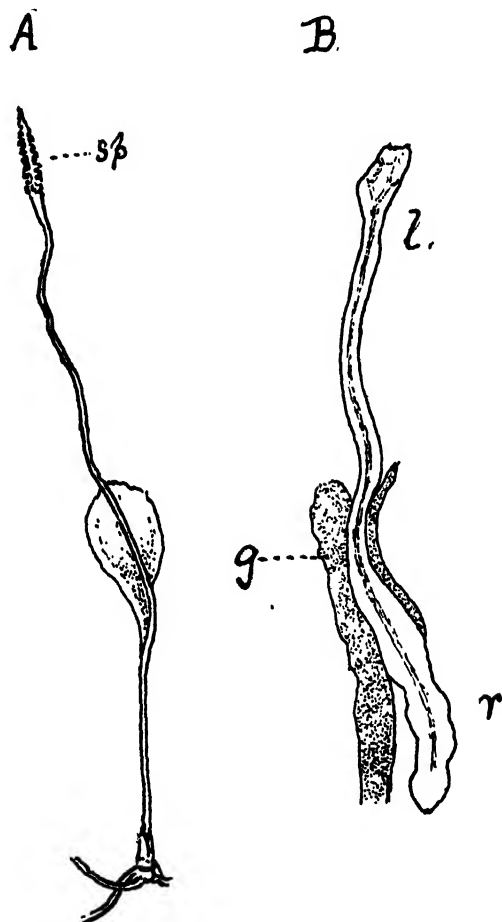
FIG. 12. A, Ophioglossum, probably the most primitive of living ferns, sp. the sporangial spike. B, longitudinal section of the young sporophyte, which consists only of the primary leaf, l, and root, r; g, the gametophyte.

much later period. However, since in *Ophioglossum moluccanum* the spike and sterile leaf segment are the results of a dichotomy of the leaf primordium, it is possible that in the beginning there was a division of a "telome" into a sporangiophore and "phylloid," such as occurs in some of the early Devonian pteridophytes. A similar dichotomy of the primordium of the fertile frond has been reported for *Botrychium lunaria*. We might also consider the possibility of the derivation of the peculiar sporangial spike of Ophioglossum from an an-

cestral type more like Anthoceros than like Rhynia. The solid spike of Ophioglossum, with its two rows of deeply sunken spore-masses, has a certain analogy, at least, with the sporogenous tissue of the Anthocerotes, where there may be quite definite alternation of fertile and sterile areas, suggesting a tendency to the segregation of the fertile sporogenous tissue into distinct masses. There might thus result a series of simple sporangia with individual marginal dehiscence, as in Ophioglossum, rather than the single terminal sporangium of the Rhynia type.

The evolution of the much branched sporangiophore of Botrychium, with its definite sporangia from the solid spike, and sunken sporangia of Ophioglossum, is readily conceivable, and this might possibly be extended to the fertile fronds of Osmunda. It is not so easy, however, to understand the development of the common fern-type with the sporangia borne on the lower side of the frond, although it must be borne in mind that, in the Osmundaceae, Todea has the sporangia on the lower surface of the leaves, much as in the common ferns.

We might perhaps imagine two types of primitive ferns developed from ancestors resembling Anthoceros; one, through intermediate forms like the Psilophtales resulting in a broad dichotomously divided frond with terminal sporangia—like Cladoxylon, the other more directly developing the spike-like sporangiophore of Ophioglossum.

That there must have been many simple fern-like plants before the end of the Devonian is indicated by the occurrence in the latter part of this era of such plants of giant dimensions, which had progressed to the point of seed production. Most remarkable of these was a genus, Eospermatopteris, discovered in eastern New York State.[10] A freshet near Gilboa exposed a great number of large stumps, some of them two feet or more in diameter. Restorations of these plants indicated that they

10 Winifred Goldring, ''The Upper Devonian Forest of Seed-ferns in Eastern New York,'' *N. Y. State Mus. Bull.*, 251. Albany, 1924.

resembled in general appearance modern tree-ferns, although differing in several important particulars. It is clear that they were not at all closely related to any modern ferns, but nevertheless, they must have had many relatives allied to true ferns.

Eospermatopteris and other fern-like plants which bore seeds are known as pteridosperms, and become a very important element in the Carboniferous floras. Many of the ''fern-fronds'' of the coal measures are now known to be leaves of pteridosperms. Some of these pteridosperms may have been the ancestors of more perfect seed plants of the later epochs. Of the very numerous fern-like fronds which abound in the Paleozoic from the Devonian onward, it is impossible always to decide whether they belong to true ferns, pteridosperms, or cycads.

The pteridosperms probably do not form a closed phylum, and there is every reason to assume that the development of seeds was attained in many independent lines. It is, therefore, not likely that all the later seed-plants are descended from the same Paleozoic ancestors.

While the seed habit was first developed in the later Devonian, it was during the Carboniferous that the pteridosperms became prominent. Seeds were also developed in some lycopods and in the peculiar order Cordaitales, whose relationships with other seed-plants are not clearly understood. Doubtfully represented in the Upper Devonian, they were abundant through the Carboniferous and Permian, but were practically extinct by the end of the Paleozoic. They were trees of considerable size, suggesting the Kauri pines (Agathis) of the Southern Hemisphere, from which, however, they differed greatly in their reproductive parts. In a way they were generalized types, showing characters suggesting the pteridosperms, conifers and cycads, but their real relationships with any of these phyla are extremely dubious.

It is in the later Carboniferous that the first recognizable ancestors of the most primitive living seed-plants appear. All the Paleozoic seed-plants, as well as their nearest living relatives, are "gymnosperms," *i.e.*, the seeds are borne either on an open leaf, sometimes resembling the foliage leaves, as in Cycas, or on scale-leaves, as in the conifers. More rarely they are borne at the apex of a naked axis—a sporangiophore like that of the primitive pteridophytes. These ancient gymnosperms evidently belong to several independent phyla, and it is clear that the existing gymnosperms represent remnants of several divergent lines of development whose relationships to each other, and to the Paleozoic seed-plants, are very imperfectly understood.

Of the living gymnosperms, two orders—Cycadales and Ginkgoales—show unmistakable evidences of fern ancestry, and presumably have originated from some Paleozoic pteridosperms. Indeed, there are many fossil leaves in the late Carboniferous and Permian which belong either directly to these two orders or to pteridosperms related to them.

Seward,[11] commenting on certain leaf-impressions resembling Ginkgo from Permio-Carboniferous beds, says, "It is difficult to resist the conclusion, though it is based on leaves alone, that this type is a Paleozoic forerunner of the group Ginkgoales." He remarks also, in regard to the cycads,[12] "The probability is these plants appeared before the end of the Carboniferous, but it was not until the latter part of the Triassic they began their rapid progress toward a position of dominance."

The Cycadales and Ginkgoales are undoubtedly the most primitive orders of living seed-plants. Not only do they recall the ferns in the character of their leaves, and especially in the cycads, in their anatomy, but, unique among living seed-plants, fertilization is effected by large ciliated sperms exactly as in typical ferns. From

[11] A. C. Seward, *loc. cit.*, p. 226.
[12] *Ibid.*, p. 281.

a study of certain fossil seeds belonging to pteridosperms and Cordaitales, it is safe to assume that in these extinct types, also, active spermatozoids were developed.

The peculiar maiden-hair tree, *Ginkgo biloba,* long cultivated in China and Japan, and sometimes planted for ornament in America, is the sole living representative of its order—a veritable living fossil. It is quite unknown outside cultivation, but has come down from remote antiquity, apparently little changed from its Paleozoic ancestors. The order was represented by many forms during the Triassic and Jurassic, which became extinct, leaving the living species as the sole survivor of the order.

Like the Ginkgoales, the cycads (Cycadophytes) attained their maximum development in the Mesozoic, especially in the later Triassic and Jurassic, when they played a very important rôle in the vegetation. While many of these were similar to the existing cycads (Cycadales), a second order, now completely extinct, contained many forms, having a more complicated type of inflorescence, which in some cases suggests the flowers of some of the modern flowering plants or angiosperms. This has led some botanists[13] to conclude that the angiosperms have been derived from some of these cycadeoids (Bennettitales).

There are, however, strong objections to this view, based in part upon fundamental differences in the floral structures, and it is hardly likely this theory will be generally accepted. These highly specialized cycadeoids reach their culmination in the Cretaceous and have no living representatives. However, the less specialized Cycadales, some of which, *e.g.,* Cycas, are probably very old types, have about a hundred living species distributed over the warmer parts of the world. In the United States two species of Zamia in southern Florida represent the order.

[13] *E.g.,* E. A. N. Arber and J. Parkin, ''On the Origin of the Angiosperms,'' *Jour. Linn. Soc. Bot.,* 38: 1907.

While the living cycads and Ginkgo are evidently relicts of a flora once much more extensive than at present, the coniferous trees, Coniferales, play a much more important rôle in the vegetation of the modern world. Over extensive areas, like western North America and parts of Europe and Asia, they constitute the major part of the forests, sometimes forming extensive stands of a single species, as in parts of the redwood belt of northern California and the Douglas fir forests of Oregon and Washington.

The earliest fossils referable to the Coniferales have been reported from Permian, or possibly late Carboniferous rocks,[14] but these primitive conifers, some of which also occur in the Triassic, are not certainly referable to any existing families, although suggesting some relationship with them. They seem to have been generalized types, in this respect recalling the Cordaitales. Among these ancient conifers may be mentioned two, Walchia and Voltzia, both of which show a possible relationship with the Araucariaceae, a family now restricted to the Southern Hemisphere. Of the two living genera, Araucaria has representatives in South America and Australasia, the most familiar species, the Norfolk Island pine, *A. excelsa,* being often cultivated. Agathis has a small number of species in Australasia and the Malayan region. The Kauri pine of New Zealand is the best known.

While remains of conifers are abundant from the Permian onward, the earlier fossils are mostly impressions of twigs and leaves, insufficient data for the determination of near relationships.

There is some evidence that conifers really related to the Auracariaceae did exist in the Permian and Triassic, and from the Jurassic onward the family was certainly established and wide-spread.

Some of the early conifers were trees of great size. In the famous Petrified Forest of Arizona, of Triassic

[14] Seward, *loc. cit.,* pp. 279–281.

Age, there are entire trunks of enormous trees, almost rivaling in size the Californian redwoods. These trunks are so perfectly preserved that the wood-structure can be clearly made out. It is said to be practically identical with that of the living species of Araucaria and Agathis. Unfortunately, nothing is known of the foliage and fructifications of these giant trees.[15]

At the present day there is a marked difference between the conifers of the Northern and Southern Hemispheres, most of the genera, and even families, being restricted to one or the other. Thus the pine family, Pinaceae or Abietineae, comprising the pines, firs, spruces, etc., is exclusively northern, and this is true of the Sequoias, bald cypresses and yews, while the Araucariaceae are entirely southern, and the Podocarpaceae predominantly so, although several species occur north of the equator, both in America and Asia. The family Callitricaceae, except for a single species, *Callitris quadrivalvis* of North Africa, is also confined to the Southern Hemisphere.

It is noteworthy that the oldest conifers show indications of possible relationships with the Araucariaceae and perhaps with the Podocarpaceae, both characteristic austral types. This suggests that they are older than the Pinaceae and Cupressineae, which possibly may have been derived from them and isolated in the north, where perhaps the development of a continental climate with its extremes of temperature may have influenced the evolution of these forms, while the more primitive Araucarian and Podocarpus types persisted in the less extreme climate of the Southern Hemisphere.

In the Jurassic, although there are abundant remains of conifers, and many examples of leaves and branches resemble closely those of living genera, the association of these with fructifications that can satisfactorily be assigned to living types is very rare. "Our knowledge

[15] Seward, *loc. cit.*, p. 305.

of Mesozoic conifers is lamentably incomplete; there are many genera and species represented by pieces of sterile foliage shoots, some bearing long and narrow leaves in two ranks, as in the yew and redwood tree (*Sequoia sempervirens*) and several others; some with crowded and more or less sickle-shaped leaves like those of *Araucaria excelsa* or Cryptomeria. It is seldom that the fossil twigs bear cones or other reproductive organs well enough preserved to be used as tests of affinity.''[16] There is, however, good reason to believe that some of them were really related to living Araucariaceae, as both foliage and cones show a marked similarity to some of the living species of Araucaria.[17]

There is strong evidence that the giant Sequoias, now confined to California, were represented in the Jurassic by closely related species, to which the name *Sequoites* has been given. Later in the Cretaceous, and especially during the Tertiary, Sequoias, identical with, or closely related to the Californian redwood, were wide-spread through the Northern Hemisphere.

In spite of the difficulty of identifying the Jurassic conifers, it is probable that all the existing families were represented—or at least the direct ancestors of these.

During the Cretaceous and Tertiary certain forms, like the Sequoias and the cypress (Taxodium) of North America, Glyptostrobus of Eastern Asia, and other forms of restricted range were abundant and during the mid-Tertiary were wide-spread through the Northern Hemisphere. Later they were replaced by pines, firs, etc., the predominant coniferous trees of the present time.

A small order of gymnosperms, the Gnetales, comprising three very diverse genera—Ephedra, Gnetum and Welwitschia—is quite unknown in a fossil condition, and their relationships are very uncertain. They are sometimes assumed to be intermediate between the other Gymnosperms, and the flowering plants, angiosperms,

[16] Seward, *loc. cit.*, p. 364.
[17] *Ibid.*, p. 363.

but this is far from being proved. Zimmermann[18] believes the order is a very old one, the few living forms being relicts of a very old group.

The Cretaceous, the last period of the Mesozoic, marks a great change in the vegetation of the world. Up to this time, to judge from the fossil record, the vegetation was made up largely of pteridophytes and gymnosperms. Suddenly, apparently—but doubtless this is more apparent than real—the plants show a decidedly modern character. The cycads and Ginkgoales and the Araucarian conifers give place to conifers of a more modern type, cypresses, redwoods, pines and firs, replacing to a great extent the archaic types of the Jurassic. ''Considering the conifers as a whole the facts which stand out most clearly are: the greater variety in genera and species in the Cretaceous than in the present northern (European) forests, and the wide distribution in the North Temperate and Arctic of types which have long been strangers in the Northern Hemisphere.''[19]

For the first time in the history of the earth's vegetation, the modern flowering plants, the angiosperms, begin to play a leading part. From the lower Cretaceous onward, they increase rapidly in numbers and importance, and soon dominate the floras of the whole world. The early history of these plants is still a mystery. Even in the oldest Cretaceous formations, where remains of angiosperms occur, they are essentially similar to forms existing to-day, and generally the lower Cretaceous angiosperms can be assigned to families, or even genera now living.

It is evident there must have been a long series of antecedent forms, presumably extending far back in geologic time, and there has naturally been much speculation as to what forms, if any, among the Paleozoic and early Mesozoic fossils, may represent the ancestors of the Cretaceous angiosperms. Some look upon the Jurassic

18 *Loc. cit.*, p. 310.
19 Seward, *loc. cit.*, p. 397.

and early Cretaceous cycadeoids, as forerunners of the modern angiosperms; others are inclined to look for their descent, directly from fern-like ancestors, possibly as far back as the Paleozoic.

While it is practically certain that true angiosperms existed during the Jurassic, and a few imperfect leaf impressions have been assigned to them, these throw very little light upon the subject. Seward[20] concludes "It is probable that the almost complete absence of fossil angiospermous leaves in Jurassic and older Mesozoic rocks is due, not to lack of flowering plants in the world but their failure to be preserved as fossils because they occupied a tract of country remote from localities where conditions were favorable for fossilization." It might be added that many of them may have been herbaceous plants whose tissues would be preserved only under especially favorable conditions.

The recent discovery of a remarkable group of Jurassic plants, Caytoniales, is of great importance, as these plants, although very different from any existing flowering plants, nevertheless have seeds borne in a closed receptacle that might be called a carpel, and there was a structure suggesting a stigma. The Caytoniales may therefore be called angiosperms, but whether or not they were related to any existing flowering plants is another question.[21]

While all the living angiosperms, sometimes called "anthophytes," are sufficiently alike in their reproductive characters to indicate real relationships, they must represent the result of evolution along many independent lines which originated at a very remote period—perhaps as far back as the Jurassic. Their still more remote ancestors were presumably of the fern-type, perhaps some form of pteridosperms, or possibly derived from

[20] Seward, *loc. cit.*, p. 366.

[21] H. H. Thomas, "The Caytoniales, a New Group of Angiospermous Plants from the Jurassic Rocks of Yorkshire." *Phil. Trans. R. Soc.*, 213: 1925.

true fern-ancestors, like the Ophioglossaceae. The microsporangia (pollen sacs) of most angiosperms are more like the ''synangia'' of the Eusporangiate ferns, or even the sporangia of Equisetum, than like the microsporangia of the pteridosperms or Cycadeoideae. Attention has been called to the similarity in the development of the young sporophyte in many monocotyledons and ferns, and especially Isoetes. It may be these indicate a real, if extremely remote relationship.

The primary division of the angiosperms into monocotyledons and dicotyledons is a somewhat artificial one, and the theory recently advanced by Engler[22] as to their relationships is probably as plausible as any that have been proposed.

Engler predicates the existence, perhaps in the Jurassic, of a wide-spread development of ''Protangiosperms,'' having most of the essential characters of the existing flowering plants. From this great complex of forms, many lines of true angiosperms developed, some monocotyledons, others dicotyledons; but he believes that neither of these gave rise to the other, and also thinks that none of the existing major divisions of the flowering plants has been derived from any of the others.

From the Cretaceous onward the development of the angiosperms has been very rapid, with a corresponding reduction in the importance of the gymnosperms. This advance has, no doubt, been greatly accelerated by the intimate association of flowering plants and insects—the two divisions of their respective sub-kingdoms now dominating the organic world.

[22] A. Engler, ''Die natürlichen Pflanzenfamilien,'' 2nd ed., Vol. 14a, 1926.

A DOMINANT MUTATION OF FREQUENT RECURRENCE IN SORGHUM

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It is generally agreed that recessive mutations occur much more frequently than dominant ones; that most recessives are unfavorable rather than favorable in their effect, and that they represent a deleterious rather than an improved condition of an organism or species. The evidence is abundant, showing that recessive mutations occur frequently in nature or may be readily induced through treatment with x-rays.

Especial interest is attached to dominant mutations for the reason that conspicuous gene changes in this direction are infrequently found among the natural mutations and are also rather rare among the mutations induced by irradiation. The types of recessive mutations found most frequently in nature are also the ones recurring the most frequently following irradiation. This appears to be true in Drosophila and in various plant species. Patterson and Muller (1930), in reporting on progressive mutations, conclude that the so-called progressive mutations can probably be produced by artificial irradiation in cases where there is the possibility of their occurring at all. They demonstrated that mutations can be produced by irradiation in both of two opposite directions at the same locus, and true reverse mutations of the forked gene were produced. As a result of irradiation, Stadler (1931) found no clear case of dominant mutation in irradiated plants, but hundreds of typical recessive mutations were produced, many of which were indistinguishable from characters previously found in nature. Dominant mutations of known and recent origin are rare, even in maize, in which more genetic characters have been

studied than any other plant species.   A case of a spontaneous dominant mutation, the ragged character, in maize is described by Brink and Senn (1931).   Teopod, a dominant character in maize, is another of the relatively few such mutations in corn which, according to Lindstrom (1925), is of recent orgin.   While both of these characters have come about through gene mutations from recessive to dominant, neither represents an improvement in the species, but rather, in effect, they are similar to many of the deleterious recessive mutations.   Horlacher and Killough (1932) report a mutation induced by irradiation from a virescent yellow recessive to a dominant normal green chlorophyll condition in cotton.

It is the purpose of this paper to describe the persistent and frequent occurrence of a dominant mutation in sorghum involving apparently only a single gene—one that has been appearing regularly during each season that the stock has been grown for the past fifteen years, and to show the rate of this mutation.

The parental stock of sorghum in which this mutation has been recurring came from a crib-run population of 652 heads of Standard Blackhull kafir grown near Chillicothe, Texas, in 1916.   Eight lots of ten heads each were selected from this population for certain head characters and planted in head rows in 1917.   These eighty heads formed the basis of eight pure lines of kafir, which have been inbred continuously for the past fifteen years and grown in field plats, comprising a total of approximately 3,000 plants each year.   Fortunately, evidence is at hand to show that this same mutation was appearing in this stock at least as early as 1916.   One of the original heads, No. 500, selected for short rachis, produced a progeny of ten tall plants, averaging 212 cm high, and four plants, normal in height, averaging 159 cm tall, which was quite close to a 3:1 ratio, considering the small population of only 14 plants.   Two heads selected from these tall plants were grown the following year, 1918, and bred true for tall.   Notes taken that season state that "several ab-

normal tall plants also appeared in the inbreeding plats which seem to indicate that the lines are not yet pure for height''; however, aside from these few plants, all the others appeared normal for height. Our recollection is that these abnormal plants continued to appear each year, more some years than others, but from one or two to five or six occurring each year among approximately 3,000 progeny from the eighty selfed heads. They constituted a source of annoyance, as the lines became homozygous for various characters under study and, with these exceptions, were remarkably uniform. It was felt that perhaps our technique in selfing was at fault and that we were getting some contamination from crossing between the various lines. In spite of precautions of bagging early and carefully the tall plants continued to appear, and since the purpose of this study with sorghums had other objectives, no attempt was made to account for these abnormalities until 1927.

Breeding Behavior of Tall Plants ·

Each year a few typical kafir plants of unusual height, considerably taller than any other forms or varieties of kafir in cultivation, continued to appear among the normal plants of the inbred lines. The tall plants are typical of the parental pure lines in which they may appear in all characteristics except height, in which respect they are from 75 to 100 cm taller than the normal plants. There appears to be no increase in the number of nodes, and the increased height is due entirely to the elongation of the internodes.

Table 1 shows the height of the tall mutations, the height of the parental line in which they arose, and the height frequency and segregation of the F_2 progeny of certain of the tall mutations grown.

Four of these abnormalities occurred in the inbred lines in 1927, two of which were planted in head-rows the following year. One of these rows segregated 36 tall to 12 normal plants, a 3:1 ratio, while the other gave 59

TABLE 1

HEIGHT OF PARENTS, TALL MUTATIONS, AND THE HEIGHT FREQUENCY AND SEGREGATION OF PROGENY OF THE MUTANTS

Line No.	Height, cm. Mutation line	Height, cm. Parent line	125	135	145	155	165	175	185	195	205	215	225	235	245	255	265	275	285	295	305	315	Total	Segregation
....	....	189				2	3	0	1	2	4				2	2	3	8	6	6	9	2	48	3:1
1	273	208				3	2	1			3	3	3	5		1			1				22	2.6:1
40	257	180			1	4	2					3	1	2	3	4							20	2.3:1
567	279	188					1	1		1	1	3	2	6	5								20	5.6:1
567	267	188				3	1	1		3	4	4	1	6									23	3.6:1
223–3	214	162		3	4	4	1	1	1	7	11	6	1										37	2.3:1
654–6	224	161	2	4	3	1	2	1	8	7	4	4	2										36	2.6:1
F₂ progenies from 3 normal F₂ plants					4	7	11	7															29	Homo.
					2	14	11	8															35	Homo.
					6	4	11	8															29	Homo.
F₃ progenies from 5 tall F₂ plants											1	4	5	7	8	10	3					38	Homo.
					2		5	2			4	5	2	6	6	5	1						38	3.2:1
						6	3				4	2	3	6	3	2	2						31	2.4:1
									9	11	14	16	7	2	1								60	2.3:1
					4	1				6	2	8	3	9	4								37	2.7:1
F₄ means of last F₃ progeny above				5	5			6	6	9	6												37	1:2:1

tall and 27 normal plants, or a ratio of 2.19:1. Heads from three of the normal recessives and five of the dominant tall plants were grown in 1929. All the recessives bred true for normal height. One of the tall plants bred true for tallness and the other four segregated tall and
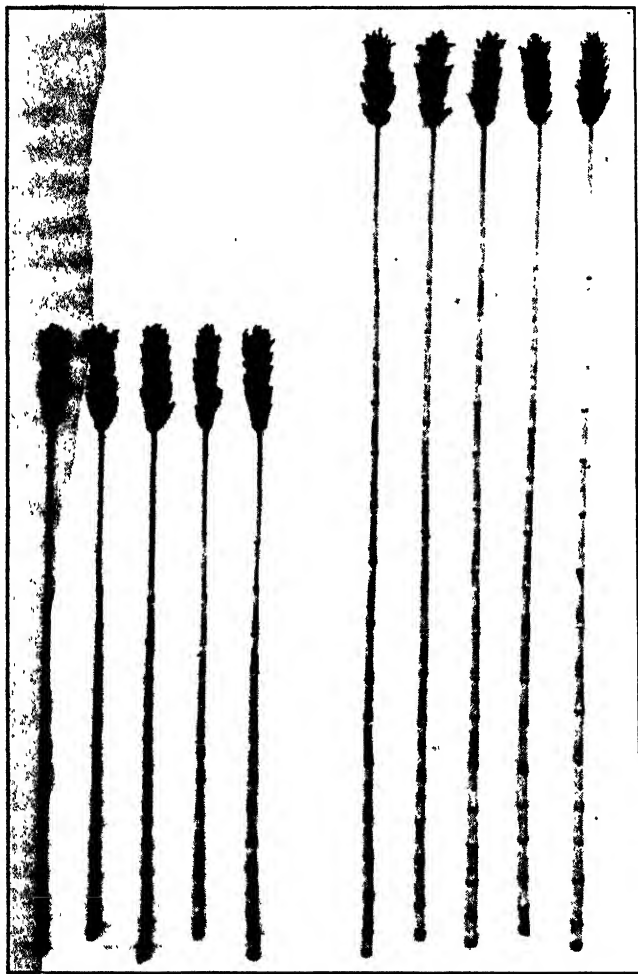
Fig. 1. Normal kafir (left) and the tall type resulting from a single dominant gene mutation (right).

normal in ratios approximating 3:1. The entire population of 37 plants from one of the latter heterozygous rows was grown into the next generation. The progeny from the ten recessives were all normal in height. From

the 27 tall plants 16 progenies segregated tall and normal and 11 bred true for the tall type. Thus the breeding behavior of these 37 plants approximated the $1:2:1$ ratio expected when only a single factor difference is involved.

Four more tall mutations appeared in the plats in 1928 and these, together with two of those occurring in 1929, were tested in the F_2 generation. All segregates fell into one of two distinct classes, normal or tall. The total population in the F_2 progenies of all the tall mutations tested during these three years has been 1,740, of which 1,310 were of the dominant mutant tall type and 430 were normal. This is a deviation of only 5 ± 12.2 from a $3:1$ ratio, and with the numbers involved, the possibility of the segregation belonging to any other Mendelian ratio is virtually eliminated. From the F_2 generation both the homozygous normal recessive and the homozygous tall mutant types have been perpetuated and found to breed true.

Tests for Effect of Crossing upon Frequency of Tall Types

In view of the fact that crossing in sorghums frequently results in marked hybrid vigor, the cross showing marked increase in height similar to the tall type plants discussed herein, it might be suspected that this new type arose through hybridization and was merely the expression of hybrid vigor. Conner and Karper (1927) have shown, however, that the marked hybrid vigor, as measured by height of plant, which accompanies the crossing of widely related sorghums, is absent when closely related forms, such as two strains or varieties of kafir, are crossed. Several of the pure lines of kafir, among which these tall mutations have been recurring, have been previously crossed also, and unpublished data on height and other measurements show a lack of observable hybrid vigor accompanying such crossing. Even though sorghums cross-pollinate readily (Karper and Conner, 1919), the eight pure lines of kafir grown in this

block of sorghums each year are fairly well isolated from other sorghums, and all the plants were carefully self-fertilized by bagging the heads before exsertion from the boot began. Under these conditions it was difficult to see how cross-fertilization could account for the regular and frequent recurrence of the abnormal tall plants.

Furthermore, the mutants are much taller—75 to 100 cm—than any normal kafir plants, and if it was assumed that they arose from seed which in some way became crossed with one of the other pure lines and, therefore, were F_1 hybrids, this would explain their presence and origin only if a hybrid had been accomplished between two of the lines which bore complementary height factors. In such event the F_2 generation would segregate in some form of a $9:3:3:1$ ratio if only two height factors were involved. As has previously been shown, however (Table 1), the F_2 segregation is simple, involving only a single gene for height.

In 1928 one of the dominant tall mutations appeared among the normal plants in each, Line No. 1 and in Line No. 40, and two of them in Line 567. In order to further eliminate the possible theory that these abnormal plants might be due to cross-fertilization, each of these three lines was intercrossed with each of the other seven pure lines of kafir in the inbreeding blocks in 1929. Eighteen combinations of crosses included all the possibilities. Pollen from one of the parental lines was dusted over the flowers of another on successive mornings as they bloomed, with the expectation of identifying the hybrids on the basis of hybrid vigor in the first generation. Previous experience with this method of crossing sorghums has shown that a considerable number of F_1 hybrids can be recovered by trimming off all the seed branches not flowering on the mornings when dusted with pollen from the plant used as the male and planting the remaining seed of the female plant the next season and identifying the F_1 hybrids through hybrid vigor, seed color, red stem or some other known character. The per-

centage of hybrids will vary with varieties used but will ordinarily be more than 10 per cent., a cross-pollination of Freeds x milo, for example, resulting in 40 per cent. F_1 hybrids appearing among the progeny the next season.

If the tall type plants are true mutations and not due to hybridization, they should occur only as frequently when the lines are intercrossed as among the inbred lines. If, on the other hand, the tall plants were the result of hybridization and the bringing together of complementary factors in the crossing process, their number should be markedly increased in the progeny of such intercrossed lines. Of course, some of the actual crossed seed would be expected to produce the tall mutant type plants, even though tallness is not due to crossing, as the result of pollen carrying the mutated gene fertilizing a normal ovule, or *vice versa*.

A population of 120 plants was grown in 1930 from each of the female parents which had been subjected to pollen from companion lines in making the eighteen combinations of crosses, and a total of four of the abnormal tall plants were present among the total progeny population of 2,160. One tall plant occurred among the progeny of the cross of Lines 40 x 223, one in Line 567 x 654 and two in Line 1 x 223. None was present in any of the other crosses. It happens that the female parents of those three crosses in which the tall plants appeared were the same as those in which the tall mutations appeared in 1928 when the parents were grown from selfed seed. There were four of the tall plants in the total population of 990 in these three lines in 1928 and the same number in a total population of 360 plants where the mother parent had been dusted with foreign pollen. The percentage of tall plants is higher than was found in the inbreeding plats but not sufficiently high to warrant the conclusion that they were due to crossing. If cross-pollination were responsible, the number of tall plants should have been increased approximately 25 times and should have con-

stituted at least 10 per cent. of the total population. Lines No. 223 and No. 654, in which mutations have previously occurred, and Line No. 153, in which they occurred later, produced no tall plants among the progeny when subjected to pollination with pollen from the other pure lines; however, a considerable number of the progeny must have been crosses, detection of which was not possible in the F_1 because of the close relationship of the parents and the lack of hybrid vigor.

These four abnormal tall plants were grown in 1931 and all segregated for height into tall and normal plants, the average ratio being $3.4:1$. Head type and other visible characters in these progeny resembled the mother stock from which these abnormal tall mutations came, except in one instance—the progeny of the tall plant arising in the population from the mother head in Line 567 that had been subjected to pollination with Line 654. In this case the characters of the pollen parent were unmistakably evident in the F_2 progeny. Line 654 is characterized by few seed branches, few nodes and other distinguishable characters, while Line 567 carries a higher number of both seed branches and nodes. Compared with the F_2 progeny of a tall mutation arising directly from the inbred Line 654, which averaged 29 seed branches and 3 nodes, the F_2 progeny of the tall mutation appearing in Line 654, when subjected to pollen from Line 567, averaged 43 seed branches and 4 nodes, intermediate between the two parents, and was segregating for these characters. Line 567 averaged 57 seed branches and 6.6 nodes to the head. It is evident, therefore, that this particular tall mutation was an actual cross between Lines 567 and 654 and that either the pollen from Line 654, or the ovule in Line 567, carried the dominant mutant gene which met with a normal one, producing a heterozygous tall plant. According to the rate of mutation in the inbred lines, this would be expected to happen about one time in 605.

Effect of the Mutant Gene on Other Plant Characters

Mutations appearing within a given pure line seem to be identical with the parent line in all observable characters except height of plant. Opportunity to definitely establish this fact was afforded in 1929 when mutations appeared in Lines No. 223 and No. 654, two inbred lines having many widely contrasting characters. Line No. 223 is characterized by having a long head, long rachis, short seed branches and many nodes in the head, while Line 654 has a shorter head, short rachis, long seed branches and few nodes in the head. These quantitative characters are quite sharply contrasted in the two lines. Crosses had previously been made between these two lines for a study of the inheritance of these quantitative characters. Although the data have not yet been published, the progenies were carried through the F_3 generation and show number of seed branches, number of nodes in the head, length of rachis and length of seed branches to be characters inherited in a simple Mendelian fashion.

Typical abnormal tall plants occurred in each of these two lines in 1929, and the difference existing between the various characters of these two mutant plants in these contrasted lines can be seen and compared with the means of normal plants of these same parental lines (Table 2). The table also affords a comparison of these characters in the F_2 generation and in the normal plants of the parental lines. The head and plant characteristics of these two mutations are very much like those of the respective parental lines from which they arose, except in height. In Line 223 the mutation had a long head, long rachis, long seed branches, many seed branches and many nodes in the head, while the mutation in Line 654 had the opposite characteristics. Further evidence of the stability of these same characters in the mutations, exactly in keeping with their expression in the pure lines

TABLE 2

COMPARISON OF CHARACTERS IN MUTATIONS, TALL SEGREGATES, AND NORMAL PLANTS FROM SAME INBRED LINES

Material	No.	Height of plant	Length of head	Length of rachis	Length of seed branches	No. of seed branches	Nodes per head	Nodes per plant
Tall mutant in Line 223 (1929)	1	214	25	20	6.1	67	8	..
Tall segregates in progeny (1930)	26	204.12 ± 1.19	23.23 ± .30	16.04 ± .40	6.46 ± .11	62.62 ± .75	7.08 ± .19	14.96 ± .09
Kafir, Line 223 (1930)	11	122.68 ± 2.09	21.45 ± .56	16.77 ± .31	6.18 ± .12	54.82 ± 1.25	5.27 ± .09	14.18 ± .15
Difference		81.44 ± 2.41	1.78 ± .64	.73 ± .51	.28 ± .16	7.80 ± 1.46	1.81 ± .21	.78 ± .17
Tall mutant in Line 654 (1929)	1	224	23	12	7.5	44	5	......
Tall segregates in progeny (1930)	26	197.58 ± 1.79	21.62 ± .36	10.65 ± .31	9.35 ± .30	30.12 ± 1.19	3.38 ± .10	14.73 ± .11
Kafir, Line 654 (1930)	12	137.83 ± 1.84	21.17 ± .49	9.50 ± .59	8.58 ± .33	26.33 ± 1.65	3.58 ± .19	13.50 ± .10
Difference		59.75 ± 2.57	.45 ± .61	1.15 ± .67	.77 ± .45	3.79 ± 2.03	.20 ± .21	1.23 ± .15

themselves, is found in a comparison of the means of the tall segregates in the F_2 generation of the mutant types and the parental lines grown the same year. The difference between height of plant in the two populations is, of course, highly significant, but all the other characters in the tall progeny are the same as in the parental lines, except number of nodes in the head and in the plant. In nodes per head the difference is more than three times the probable error in Line 223, but there was no significant difference in this character in Line 654. Number of nodes per plant shows a difference significant in relation to its probable error in both lines. The largest difference is in Line 654 and is undoubtedly due to the small number in the population of the parental line and failure to get a random sample of measurement of this character. Further, the relative constancy of number of nodes per plant results in a low standard deviation of the mean, a low probable error, tending to exaggerate the significance of a small difference. Actual average number of nodes common to this line over the past five years was 14.9, or more than one node above the mean obtained from the sample used in this table, so that the significant difference between the population with respect to this character is more apparent than real.

Means of the F_2 progenies were calculated separately for the normal and tall segregates and show a very close similarity of both classes for all characters except height of plant, the only one differing significantly. Thus the tall plants are recovered in the F_2 generation identical with the original mutation and having all the attributes of the parental line from which it arose except height of plant, for which apparently only a single gene is responsible.

RATE OF MUTATION

Before trying to analyze the data so as to arrive at conclusions regarding the rate of mutation, several important points should be disposed of. The eight pure lines

of sorghum, each with a population of 330 plants grown every year, comprised a total population of 2,904 inbred plants annually, and tall mutations have occurred in six of these eight lines in the past four years (Table 3). In-

TABLE 3

RATE OF DOMINANT TALL MUTATIONS WITHIN EIGHT PURE LINES OF SORGHUMS, 1927–31

Year	Line No.	No. of mutations	Total population	Mutation ratio	
				Zygotes	Gametes
1927	All	4	2,904	1: 725	1: 1451
1928	1	1	330	1: 329	1: 659
1928	40	1	330	1: 329	1: 659
1928	567	2	330	1: 164	1: 329
1928	Composite	1	264	1: 263	1: 527
1928	All	5	2,904	1: 580	1: 1161
1929	1	1	330	1: 329	1: 659
1929	223	2	330	1: 164	1: 329
1929	654	1	330	1: 329	1: 659
1929	All	4	2,904	1: 725	1: 1451
1930	1	1	330	1: 329	1: 659
1930	Composite	1	264	1: 263	1: 527
1930	All	2	2,904	1: 1451	1: 2903
1931	1	2	330	1: 164	1: 329
1931	153	1	330	1: 329	1: 659
1931	223	2	330	1: 164	1: 329
1931	567	1	330	1: 329	1: 659
1931	654	1	330	1: 329	1: 659
1931	Composite	2	264	1: 131	1: 263
1931	All	9	2,904	1: 322	1: 644
Total	All	24	14,520	1: 604	1: 1209

cluded in this total each year, also, is a population of 264 plants in check plats grown from massed seed of the eight lines. Mutations have been recovered more frequently in some lines than in others, as, for instance, in Line 1 the mutations have appeared every year, whereas, in Lines 192 and 646 none has occurred in the four-year period. In Line 646, however, a tall plant, apparently identical with the mutations found in the other lines, did appear in 1930 among the progeny of 500 seed treated

with x-rays. Whether this same gene mutation was in-
duced by radiation or was already present in the stock
before treatment is not known, but the latter would seem
the more likely. Only one mutation has appeared in
Line 40, while five have been found in Line 1. Is this
difference significant and is the mutation rate of Line 1
different from Line 192, in which no tall plants have been
found, or can we combine all the lines into one population
in considering the rate of mutation?

If we assume that 1:604, the proportion of mutations
found in the total population of all eight strains, is the
true, or approximately true, rate of mutation, we may
consider the probabilities of the various possible propor-
tions of mutations to be represented by the expansion of
the binomial $\frac{1}{605} + \frac{604}{605}$. The probability of getting any
given number of mutations in a sample of 1,320, the num-
ber grown in each line during these four years, may be
found by expanding $(\frac{1}{605} + \frac{604}{605})^{1320}$. By partial expansion
of this binomial the probabilities of getting from 0 to 10
mutations in a sample of 1,320 have been computed.

It is found that the probability of getting no mutations
in a sample of 1,320 is .11, which is equivalent to odds of
8.1 to 1 against the possibility of getting no mutations in
the population of 1,320 plants, the number involved in
Lines 192 or 646, respectively.

The probability of getting 5 mutations in a sample of
1,320, assuming 1:604 as the true rate of mutation, is
.05, representing odds of 19 to 1 against such occurrence
due to chance. The probability of getting 5 or more
mutations in a sample of 1,320 is .07, representing odds
of only 13.3 to 1 against such occurrence. Apparently
Lines 1, 40 and 192, in which five, one and no mutations,
respectively, have occurred, need not be considered as
differing significantly in rate of mutation.

In each of the five years the total number of plants
grown from the eight lines was 2,904, but the number of
mutations observed in this population varied from two to

nine. Is this a seasonal difference or is it a chance fluctuation? By the expansion of the binomial used above to the power 2,904 we find that the probability of getting two mutations, the smallest number obtained in any one year, is .10, which is equivalent to odds of only 10 to 1 against such occurrence, while the probability of getting two or less is .14, representing odds of 6.1 to 1 against such an occurrence in a sample of 2,904. The probability of getting nine mutations in a sample of this size is .03, representing odds of 32.3 to 1 against such occurrence; however, the probability of getting nine or more mutations in such sample is .05, representing odds of only 19 to 1 against such occurrence due to chance.

It is seen from these probabilities that the distributions discussed above are all within the limits of chance variation, if the rate of mutation is 1:604, and it appears that grouping all lines and all years into one population is justified.

The total combined population of the eight inbred lines grown during the past five years was 14,520 plants, and a total of 24 tall mutations appeared during this period (Table 3). The mutation ratio, considered on the basis of zygotes, is 1 to 604. Presuming that the mutation of this gene may occur as readily among the male gametes as among the female gametes, the mutation ratio in the gametes is 1 to 1,209, or 827 per million.

DISCUSSION

Since this tall character is clearly a dominant, it could not be carried along as a latent character, and its recurrence is undoubtedly the result of frequent gene mutation. The mutation likely occurs during gametogenesis of the parental plant, the generation prior to the appearance of the tall plants, where a gamete carrying the mutated gene unites with a normal gamete and produces the seed from which the heterozygous mutant plant develops. A normal ovule fertilized by pollen carrying the mutant gene, or an ovule carrying the mutation fer-

tilized by normal pollen, would explain the hybrid condition and simple segregation in the progeny of the tall plants.

It is quite improbable that the abnormal tall type plants are the result of abnormal chromosome behavior because of the simple hereditary performance of their progeny. If a chromosomal aberration is responsible, and a whole section of a chromosome rather than a single gene is involved, then the factors for number and length of seed branches, length of rachis and number of nodes in the head must be located on another chromosome than the one involved with the tall type, because these characters appear to segregate normally in the progeny of the mutant type plants.

If the tall plants were the result of a bud mutation occurring in a somatic cell somewhere in the ontogeny of the plant, except tissue just previous to that concerned in pollen mother cell development, a certain area of considerable size should sometimes be affected and instead of one seed, or a very few seed, on the head producing a tall plant, a considerable number of such plants should result. Furthermore, such an area on the panicle, resulting from the outgrowth of a somatic cell in which the gene had mutated from normal to tall, would be heterozygous, producing two kinds of gametes, T and t, and one out of four of the seed set should produce a TT plant that would breed true for tall. The new tall type is dominant, segregates clearly from the normal, and one third of them breed true. All the tall mutations tested have segregated 3:1 in the succeeding generation. Of course, there is still the possibility that a somatic mutation occurred just previous to reduction division but so late in development that it affected only the pollen mother cells or the megaspore mother cells but not both. The results of such a somatic mutation would be substantially the same as one occurring during reduction division, except that a mutation just previous to reduction division might be expected to cause a number of the mutants to

appear within the progeny of a single family. It is true that on four occasions there have been as many as two mutants among the progeny of a single line, while other lines showed none. The probability of finding two or more mutants in a population of 330 plants, from material mutating at the rate of 1:604, is .10 and would be expected to occur once in ten trials. However, two mutants appearing in a single line actually occurred four times out of twelve trials, which is rather frequent on the basis of the probabilities involved. If the two mutants observed in each of these instances were considered as the result of only a single mutation the total number in the four years would be reduced from 24 to 20 and the rate of mutation would be 1:726 zygotes instead of 1:604, the rate when each mutant plant is regarded as a separate mutation.

It appears quite certain that we are dealing here with a single dominant gene which increases the height of the plant approximately 40 per cent., increases the production of the plant, and can be definitely classed as a favorable mutation in the biological sense. Economically, this new type is superior from the standpoint of forage and silage and is being tested and distributed for this purpose. From the standpoint of convenience in harvesting for grain, however, it would be considered inferior to the parental types, as pointed out later.

Not only is this gene change a dominant and favorable one, but there is evidence that the mutation has been recurring under natural field conditions, at least during the past 15 years, and the rate of mutation during the past four years has been very high when compared with the mutation rate of genes studied in other species. Stadler (1931), in studying the frequency of recessive mutations in eight well-known genes in maize, found the most mutable gene yielded about 400 mutations per million gametes. In this gene for height of plant in sorghum, the rate of mutation was 827 per million gametes, or about twice as great as the most mutable

gene among the recessives studied in maize. Although the specific dominant tall gene in sorghum is highly mutable, a number of recessive gene mutations have been found much less frequently in these same inbred lines of kafir (Karper and Conner, 1931). During the past 15 years two recessive gene mutations affecting chlorophyll development have occurred in each of three of the inbred lines, while none has appeared in the other five inbred lines. While accurate data on the rate of mutation for any one of the specific chlorophyll deficient genes are lacking, the rate of these more or less common recessive mutations is certainly very low, when compared with the frequent recurrence of the dominant mutation discussed in this paper.

In the sorghums we find what appears to be a parallel series of height variations common to a number of the subspecies or varieties. For example, in each of the kafir, milo, feterita, kaoliang and broomcorn groups we have strains or varieties known as Standard, Dwarf and Extra Dwarf. The original introduction of these subspecies of sorghum was practically all the Standard or taller types, and the Dwarfs and Extra Dwarfs originated, for the most part, by mutation in this country during the past two or three decades and have very largely replaced the tall or Standard types originally imported from Africa, largely because of the convenience in harvesting and not because they are more productive. Data on the inheritance of height in this series of plant statures indicate that a single factor is responsible for height difference between Standard and Dwarf and another factor responsible for the difference between the Dwarf and Extra Dwarf series. These single genes responsible for height differences in sorghum are illustrative of the vast economic potentialities that may be packed in a single recessive gene. Prior to 1906, all the milo grown in the United States was of the tall or Standard variety. About that time Dwarf milo suddenly

appeared, undoubtedly through a natural recessive muta-tion of a single gene. Its desirable characteristic was recognized, the seed supply increased, and the new type soon replaced practically all the Standard variety. To-day, at least 50 million bushels of grain, annually, are grown from Dwarf milo, a simple recessive from Stand-ard milo and differing only in height of plant, which has certain advantages in harvesting as a grain crop.

The tall mutation described here is in the direction of increased height and a dominant representing a stature above the common Standard kafir but differing from it only by a single gene. Although this new mutant type is not common to the sorghums in this country, the for-eign introductions, which have undergone little selection, are generally tall-growing types, so that this tall mutant type is probably present among the native sorghums in Africa and India, and the specific gene mutation de-scribed here may be a reversion to the wild type.

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NOTES ON FEEDING AND MOLTING IN FROGS[1]

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INTRODUCTION

FOR a number of years the writer has been working, by the customary dissection method, on the food of frogs of the eastern United States. Supplementary and somewhat preliminary to this, a few experiments have been conducted in hopes that a better understanding of the food of frogs might be obtained.

FEEDING

Experiments on the feeding of frogs are not numerous, although much dissection work has been done to determine stomach contents. One of the most complete records of the diet of an individual frog appears in D. C. Beard's "American Boy's Handy Book." He itemizes the daily diet of a frog, probably *Rana catesbeiana,* from May 14 to November 17. During this period, the frog consumed 12 beetles, 9 mice, 1 frog, 3 crawfish, 1 bat, two thirds of a perch, and, in addition, tackled a young alligator 11¾ inches long. The latter was disgorged by force. Miller (1909) observed that a toad took ninety to one hundred rose beetles at a single feeding. In the report of the Porto Rico Experiment Station (1926) we learn that a Surinam toad will eat nearly 10,000 injurious insects in three months.

Robert Matheson, a twelve-year-old boy of Ithaca, N. Y., amused himself and friends one evening by feeding fireflies (Lampyridae) to a frog. The fireflies emitted their cold light through the thin skin of the cold-blooded animal.

[1] Read before the American Society of Zoologists at New Orleans, December 30, 1931.

Feeding experiments were conducted in the following manner. Individual frogs were placed in battery jars with about an inch of clear water in the bottom. The tops of the jars were covered with cheesecloth to prevent undesirable insects from entering. Every morning, the ejected pellets and cast skins, when present, were removed and the frogs were transferred to clean jars. At the same time, live food, chiefly spiders and insects, was introduced. The water served to keep the frogs moist and facilitated in securing the molted skins. Records were kept of the food offered to the frogs, the recovery of such food in the feces and dates when molts occurred (see Tables 3 and 6).

It is evident from Table 1 that the frogs were underfed rather than stuffed. If it be true, as some writers state, that a toad will take 10,000 injurious insects in three months, the frogs represented below must have been on a diet.

TABLE 1

QUANTITY OF FOOD TAKEN BY FROGS

Species	Sex	Size	Insects offered	Insects eaten	Insects refused	Duration of experiment
Rana sylvatica	♀	1¼″	182	106	77	Aug. 24–Oct. 21
Rana clamitans	♀	4″	335	286	49	June 12–Oct. 29
Rana clamitans	♀	3½″	65	43	22	Sept. 23–Oct. 28

The maximum amount of food taken by any of the frogs mentioned in Table 1 occurred on October 5, when *Rana clamitans* accepted fifteen insects, then refused to take more. Table 2 gives the menu for this occasion.

Rate of digestion: Concerning digestion, Holmes (1927) states that it is comparatively slow in frogs and the time varies with the amount of food accepted. Langlen (1881) observed that earthworms require somewhat less than twenty-four hours to digest, but if several are given, they do not disappear from the stomach until a longer period.

TABLE 2

MAXIMUM CAPACITY OF *Rana clamitans*[2]

Time food offered	Nature of food	Manner of accepting food
1 P. M.	3 Grasshoppers 1 Xylocopa	Instantly "
3 : 30 P. M.	1 Xylocopa	1 hour later
3 : 45 P. M.	1 Syrphus fly 1 Spider 1 Moth	Instantly " "
4 : 15 P. M. ..	3 Spiders 1 Fly 1 Bee	Instantly " "
4 : 45 P. M.	1 Spider 1 Vespa	Instantly Later in evening

[2] This four-inch frog took two flies the preceding day. The following day nine insects were offered, but only seven were accepted.

The duration of the digestive process, or better stated here, the time required for food to traverse the alimentary canal, was determined by feeding insects to a frog and later recovering these insects or parts of them in the excrement. One kind of food, for example, arachnids, was fed for several days, then a beetle or a wasp was offered. These insects could readily be detected in the feces, but much food had to be fed in order to trace a single species. This method of experimentation may have certain disadvantages, for the frog is subjected to unnatural conditions, especially the lack of exercise. A sample record sheet from laboratory notes is given in Table 3. The interception of several insects is shown, especially *Anasa tristis,* the common squash bug, and a Tipulid, which give positive records.

Table 4 is a summary of insects recovered in the feces of three different frogs, two *Rana clamitans* and one *Rana sylvatica*. The species showed little difference in the rate food passed through the alimentary canal. It is apparent that the stomach must be emptied on the average of once in two or three days. The rate is more

TABLE 3

A Part of the Record of *Rana clamitans*. ♀, 4 Inches Long

Date	Insects offered	Parts of insects recovered in defecations		
		Heads	Thoraces	Wings
June 29 ..	5 Muscids 1 Tenebrionid 1 Honeybee (*1 Anasa*)	3 Hymenoptera	2 Sphecius	3 Hymenoptera
30 ..	12 Muscids	3 Hymenoptera	1 Diptera	2 Sphecius 2 Aphids 4 Muscids 1 Hymenoptera
July 1	6 Muscids 1 Noctuid 1 Caterpillar 1 Chalybion 2 Sceliphron	(*1 Anasa*) 2 Diptera 2 Coleoptera	(*1 Anasa*) 2 Tenebrionids	(*4 Anasa*) 9 Muscids 2 Coleoptera 8 Hymenoptera
2	6 Muscids (*1 Tipulid*)	1 Sceliphron 7 Diptera	1 Chalybion	13 Diptera 1 Sceliphron
3 ...	2 Tenebrionids 4 Muscids	7 Muscids 1 Sceliphron	1 Sceliphron	6 Sceliphron 4 Schalybion
4 .	1 Chalybion 1 Calopteryx	6 Muscids 1 Chalybion (*1 Tipulid*)	(*1 Tipulid*) 1 Sceliphron	2 Sceliphron (*2 Tipulidae*) 5 Muscidae
5 .	Nothing			1 Chalybion
6	4 Sceliphron 1 Muscid 1 Bombus			
7	1 Honeybee 5 Snails 1 Carabid	1 Chalybion 2 Tenebrionids 1 Muscid	1 Sceliphron 1 Muscid 1 Tenebrionid	Calopteryx 4 Tenebrionids 4 Sceliphron 4 Muscids

rapid if food is abundant and slower if the stomach is not full. When the alimentary canal is crowded with food, digestion is not complete and large parts of insects, often whole insects are voided, but when food is limited, digestion is more complete and insects are frequently digested beyond recognition. Portions of a few insects were voided the same day they were fed, but the majority of the insects did not appear in the feces until one or two days after the frog accepted the food. In one case, parts of a beetle were recovered seventeen days later.

TABLE 4

TIME REQUIRED FOR FOOD TO TRAVERSE THE ALIMENTARY CANAL OF FROGS

Days after feeding to frogs	½	1	2	3	4	5	6	7	8	9	10	17
Number of insects[3] recovered from frogs	3	66	61	43	27	8	8	4	4	3	1	1

Not all food taken into the mouth of frogs passes through the alimentary canal. Gadow and Strickland (1909) relate the interesting habits of an Australian Hyla that vomited the shells of snails taken the preceding day. Undesirable parts of insects are cast aside before swallowing. I have seen frogs break off the tips of the wings of *Calopteryx maculata* and cast them aside. Sticks and stones are sometimes found in the stomach, but they are often discarded before passing the mouth. If a bee or wasp stings, after it is taken into the mouth, the frog quickly rejects it. The sensation may persist after the insect is expelled, but the frog continues to evert its tongue for several minutes as though the insect was still present.

In swallowing, the frog frequently goes through considerable contortions. The head is lowered, the eyes are depressed within their sockets and the frog often uses its feet to adjust or push the food down into the stomach.

Slow-moving objects, as snails, attract the attention of hungry frogs as well as faster insects, for instance, bees and wasps. A quick raising of the head or a right-about turn may indicate that the frog is aware of the presence of food. Their bulging eyes permit them to discern objects almost in back of them.

Feeding continues throughout the summer at a uniform rate except when food is not abundant, when the frog becomes full or possibly slackens during molting. The writer has observed them feeding even in the midst of a molt.

[3] This includes only insects or parts of insects that could be definitely traced.

MOLTING

Writers of Amphibia mention briefly that molting occurs in transformed frogs periodically at varying intervals, usually about once a month. None except Knauer (1879) give detailed figures on the molting process. He described molting in reptiles and Amphibia and we reproduce herewith the portion dealing with toads.

FIG. 1.   A series of frogs under observation to determine feeding and molting habits.

TABLE 5

MOLTING IN AMPHIBIA AFTER KNAUER 1879

Species	Sex	March	April	May	June	July	Duration
Bufo vulgaris	old ♀	18	19	17	20	18	at most an hour
Bufo vulgaris ...	old ♀	24	26	23	24	25	at most an hour
Bufo vulgaris	young ♂	22	24	25	24	26	at most an hour
Bufo variabilis	♀		19	21	25	23	brief
Bufo variabilis	♂		24	26	29	30	brief

Fischer-Sigwart (1897) state that *Rana fusca* has monthly molts: the first occurs from late February to early April; the second the latter part of May to the first of June; the third in July and the fourth in August. Wilder (1925) has shown that the mechanism which sets in action the molting process may stimulate a series of molts in rapid succession. Adolph and Collins (1925) believe that a chemical action starts the molting process, while Adams, Richards and Kuder (1930) show that the

thyroid and pituitary glands play a part in the molting of Triturus. Holmes (1927) and Noble (1931) treat of the molting process generally. The writer adds a few original records of the molting habits of four North American species.

Table 6 shows little or no tendency to monthly molts as the general rule in Amphibia. Molts are numerous and frequently prolonged for three or four days. (While the table indicates periods of four-day molts, laboratory notes show that molts frequently started in the afternoon and terminated in the morning, so that molts seldom

Fig. 2. Type of jar for studying feeding and molting.

TABLE 6
MOLTING RECORDS OF FROGS

Species	Sept.	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Rana clamitans				×		×	×	×						×		×		×	×					×			×	×		
Rana pipiens	.	×	×			×	×					×						×						×				×		
Rana sylvatica		×	×		×			×	×							×						×	×		×	×	×	×	×	
Rana sylvatica						×																×	×				×		×	
Bufo americanus	×															×	×		.								×	×	×	

Species	Oct.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Rana clamitans			×	×								×	×			×	×		×	×			×				×	×				×
Rana pipiens		×											×								×		×				×	×		×	×	
Rana sylvatica				×								×	×			×					×						×	×	×			
Rana sylvatica	.			×					×			×		×				×	×	×				×								
Bufo americanus														×	×	×	×	×	×		×									×	×	×

Species	Nov.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Rana clamitans	×		×	×				×	×	×					×					×	×							×	×		
Rana pipiens			×	×		×				×					×					×	×				×			×			
Rana sylvatica				×				×						×			×	×								×	×				
Rana sylvatica																×	×														
Bufo americanus	×	×							×	×	×				×	×	×	×													

Species	Dec.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Rana clamitans			×					×																			×	
Rana pipiens	.				×											×				×								
Rana sylvatica			×	×													×							×				
Rana sylvatica													×									×						
Bufo americanus			×	×	×																							

exceeded seventy-two hours.)   Resting periods between molts are not clear-cut, except in the case of *Bufo americanus.*   In the toad, there is a tendency towards molts twice a month, with distinct resting periods between and molts extending over three or four days.   When cold weather approaches, the molting process slackens.   During November and December, the frogs under observation were kept indoors where the temperature ranged between 40 and 70 degrees F., and we find an interesting prolongation of the molting process almost to the end of December.

Feeding, as noted before, generally ceases during the shedding of the skin, but it is not unusual for a frog to take food during this time, if the skin is not loose about the mouth.   It is questionable whether there is a direct correlation between feeding and molting.   Frogs under observation molted frequently but were at no time overfed.   Molting also occurred in *Hyla pickeringii* early in spring before feeding commenced.   It is furthermore known that starving newts and other animals molt.

There seems to be some diversity of opinion whether frogs eat their molted skins.   Cunningham (1912) and Noble (1931) state that many frogs and salamanders eat their newly shed skin.   The writer has observed numerous molts in *Rana sylvatica, Rana clamitans* and *Bufo lentiginosus americanus,* but has never seen these species eat their molted skins.   Dissections show that *Hyla pickeringii* often eats its first molt in early spring.   A series of thirty-five specimens of *H. pickeringii* were collected at Arendtsville, Pennsylvania, on April 5.   They had apparently emerged from their hibernation quarters very recently and probably took trash and their molted skins in the absence, at this time, of more nutritious food.   Trematodes, a part of the fauna of the intestine of this species, were found in abundance, sometimes as many as twenty-five in a single frog.

As to the manner of shedding the skin, a variety of opinions exist.   Simpson (1913) states that newts shed

TABLE 7

CONTENTS OF THE ALIMENTARY CANAL OF 35 *Hyla pickeringii* COLLECTED
APRIL 5, 1923

Contents of alimentary canal	Empty	Insect food	Foreign material	Molted skins	Trematodes
Number of frogs ..	2	4	9	7	26

their skin in one piece, Wright (1920) believes that the toad and the salamander shed in one piece. The writer is of the opinion that toads and frogs as a rule shed their skin in several pieces.

In closing, it might be well to state that although salamanders, frogs and toads are closely related, their feeding and molting habits apparently differ.

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A CONTRIBUTION TO THE ECOLOGY OF THE SALT MARSH SNAIL, *MELAMPUS BIDENTATUS* SAY

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THE salt marsh snail (*Melampus lineatus* or *bidentatus,* Fig. 1) is a minute species, one of the smallest of our
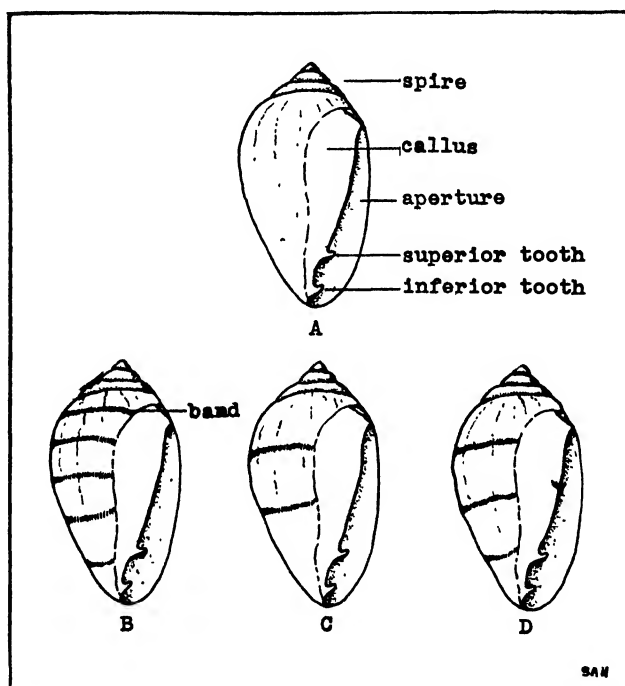
FIG. 1.   The salt-marsh snail (*Melampus bidentatus*). A. Typical shell of adult snail, lacking the transverse bands. B, C, and D. Shells of young snails, showing typical variability in banding.

native *Gasteropoda,* being nine to twelve millimeters in length when fully grown. It occurs all along the Atlantic Coast, and is the commonest form in salt marshes and tidal estuaries, being found in both salt and brackish waters, and extending from Massachusetts to Florida and along the shores of the Gulf of Mexico as far as

Texas.   It occurs most abundantly in the New England portion of its range.

Very little variation occurs in the shape of the shells in this species, which is ovoid.   The much-compressed spire consists of three small whorls; a fourth and largest whorl comprises almost the entire bulk of the shell.   The slit-like aperture, narrower at the posterior than at the anterior end, is about three fourths the length of the entire shell.

A considerable latitude, however, is found in the coloration and the banding.   The color ranges from a pale yellow through a light brown to a darker brown, to olive brown, sometimes reaching nearly to a black.   The transverse bands may be absent in adults, but present, in numbers from one to six or seven, in the young.

The aperture of the shell is bordered by a white, smooth area which is called the callus.   Two prominent, whitish ridges which appear like teeth (superior and inferior tooth) when viewed ventrally, are present on the inner wall of the aperture.

The following account of the species is drawn from observations made in the vicinity of New London and New Haven, Connecticut, during 1930 and 1931.

The typical local distribution of *Melampus* in a salt marsh is shown in Fig. 2.   From the zone between the
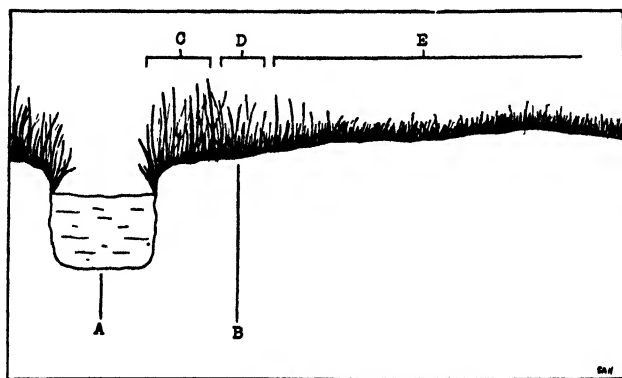
Fig. 2.   Typical distribution of *Melampus bidentatus* in a salt marsh. A. Tidal creek.   B. High tide line.   C. Zone of coarse marsh grass.   D. Zone of mingled coarse and fine marsh grass.   E. Salt marsh itself (area of fine marsh grass).

low and high tide lines, Zone C, where the water rises to submerge the tall marsh grass, and where Fiddler Crabs (*Uca*) are usually numerous, *Melampus* occurs but sparsely. They are few or absent in Zone D, and begin to be numerous in the salt marsh above the point of the high tide line, B.

Although salt marsh snails are nocturnal feeders, some individuals emerge and may be found on the mud and the stems of grasses during the day, in shaded situations. Some few crawl about in the sunlight, but as a rule the direct rays of the sun are avoided. On damp, foggy or cloudy days many more of the snails become active, perhaps a quarter of the population of a given area. The majority, however, during the daylight hours, secrete themselves under stones, shells, bits of débris, or in dense tufts of marsh grass, and there remain quiescent with the body retracted far within the shell. About an hour after sunset they begin to emerge onto the bare mud areas in the marsh, which are covered with a thin greenish-brown slimy deposit. They also crawl up along the stems of the marsh grass, and over bits of débris, if these bear the same greenish-brown film.

During these periods of feeding the snails crawl slowly along, gathering in the slimy deposit just mentioned. Microscopic examination of this deposit, in the neighborhood of feeding individuals, showed it to be composed of diatoms, filamentous green algae and *Oscillatoria,* together with a gelatinous substance, and such fragments of the epidermal cells of the grasses as were being sloughed off or decayed.

Microscopic examination of the stomach contents of actively feeding individuals showed a large quantity of these epidermal cell fragments, together with bits of the filamentous algae noted before, and diatoms. Examination likewise of freshly voided excrement from feeding individuals (which appeared in minute vermiform masses on the mud and grass stems) exhibited fragments of the

same substances.   From this, the food of the species is clear.

The eggs of the species (Fig. 3) are deposited on broken twigs, small stones, shells and grasses; and are
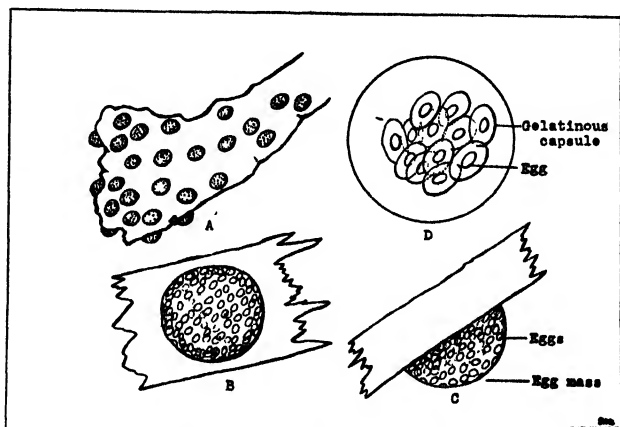
FIG. 3.   Egg masses and eggs of *Melampus bidentatus*.   A. Egg masses *in situ* on a broken twig found lying on the mud.   B and C. Enlarged views of egg masses.   D. Magnified portion of egg mass, showing the eggs.

laid in convex masses, each mass containing about two hundred eggs closely packed together in a transparent gelatinous matrix.   The average major diameter of the egg masses is from one to two millimeters.

During the winter months the animals go into hibernation (though a few individuals may be found active during warm periods).   While lying dormant during this season, the snails are closely packed together underneath shells, stones, dense tufts of marsh grass and holes in the mud.   In such periods of inactivity the body is retracted far into the shell.   The hibernating localities are well above the high tide line in the region of the short marsh grass, often on little hillocks in the marsh.

Melampus is preyed upon, and forms an important item in the dietary of small fishes, such as *Fundulus*. The stomach of *Fundulus pisculentus* caught at Woods Hole in July by Verrill contained no other food except large numbers of these snails.   *Melampus* is also eaten by various marsh and aquatic birds, and also by song

sparrows, marsh wrens, swamp sparrows, red-winged blackbirds and other marsh-inhabiting species.

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COLOR AND PRIMENESS IN VARIABLE MAMMALS

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The phenomenon of seasonal color change commonly seen in northern animals became of particular interest to the writer when it was found that primeness in all fur-bearing animals, save the albino, was solely dependent upon a blanching process of the hair-roots and entirely independent of dermal thickness or pigmentation. Subsequent investigation showed that the depigmentation process did not cease at the level of the epidermis, but that it was continued out into the proximal portion of the hair-shaft to a variable extent in different fur-bearing animals. The latter fact suggested the idea that the blanching of the jack-rabbit and Arctic white fox might merely be the outward evidence of an exaggerated state of this same physiological process.

Examination of the literature gave a very wide range of opinion as to the cause and nature of the seasonal change of color in these mammals. One interpretation of the process is that expressed by Allen (1894), Anthony (1928), Seaton (1928), Nelson (1918), and Pennant (1784), who with one accord state that the autumnal change of color in variable mammals (*Lepus americanus*) is due to a shedding of the pigmented summer fur and the growth of a white (albinotic) winter coat. On the other hand, Richardson (1829), Hadwen (1929), Merriam (1884) and Welch (1869) suggest that the change is not due to a shedding of the summer pelage, but that the existing coat lengthens and undergoes a blanching process, thus providing the winter coloration.

Both sides, with the exception of Hadwen and Merriam, apparently agree as to the manner in which the reversion to summer dress takes place, namely, that the

white winter fur is subsequently shed and replaced by the pigmented summer coat in the spring season.

The controversy as to the manner in which the process takes place has been waged over a century and a half, and the following extracts from the above authors indicate the opinions held by those on each side. Allen (1894) states:

(1) that the change of colour, both in autumn and in spring, is due to a change of pelage, and not, even in the fall, to a change in color in the hair itself. (2) Further, that this change is gradual, occupying many weeks, both in fall and in spring, and that while it may be doubtless more or less accelerated or retarded by temporary climatic conditions, it is not intimately connected with phases of weather, but is as regularly periodic as the seasons themselves. (3) That the method of change, as regards the parts first affected, is the reverse in spring of the order characterizing the autumnal change; in the fall the change beginning with the feet and ears, the sides of the nose and front of the head, which often become radically changed before the body is much affected; while, as regards the body, the change begins first at the base of the tail, and extreme posterior part of the body, working thence upwards toward the median line of the back and from behind anteriorly, the crown of the head and a narrow median line over the shoulders and front part of the back being the parts last changed. In the spring the order of change *is exactly the reverse,* the moult beginning on the head and along the median line of the anterior half of the dorsal region extending laterally and gradually to the ventral border of the sides of the body and posteriorly to the rump, and then later to the ears and down the limbs to the feet, which are the parts last affected, and which often remain but very little changed till the head and body have pretty completely assumed the summer dress.

Opposing this view Merriam (1884) states:

For in the fall the change certainly does occur, by a lengthening and blanching of the summer fur, the individual hairs changing color after the first fall of snow. This species, like the great majority of mammals, is clothed with two kinds of hair—a fine soft fur which densely covers all parts of the body, and longer, stiffer hairs, scattered through, and projecting beyond, the former. These long hairs are black in summer and white in winter. In the fall of the year when the change begins, they become white at the tips first, the black gradually fading from above downwards until the entire hair is white.

In the spring the process is reversed, the exposed portion of the long hair becoming black (though the extreme tips sometimes remain white until the change is far advanced), which color gradually extends downwards, at the expense of the white, until the entire hair is black. Sometimes the displacement of the white is temporarily interrupted, the two colors appear-

ing in alternate zones, and, during the latter part of March, when the body of the animal is still white, it is not uncommon to find hundreds of black hairs scattered over the back, many of them with extreme apices, and a narrow zone between the middle and base, white. In fall or early winter the soft fur becomes tipped with white, the white portion increasing somewhat in length and diameter. In spring a curious phenomenon takes place. The white portion of the fur loses its vitality, becoming brittle, and breaks off on slight friction, so that the animal in brushing through the undergrowth soon rids itself of it. As a rule the long hairs change first. Both in spring and fall the time of change seems to be governed by the presence or absence of snow and is not affected by temperature. It occurs independently of the moult, and the new hairs assume the prevailing color of the animal or the color towards which it is tending at the time of their appearance.

Probably the main reason for the great difference of opinion is due to the difficulty of trying to interpret all the conflicting phenomena proceeding simultaneously in these variable animals, which mask the more fundamental processes that are clearly seen when studying a pigmented non-variable animal such as the muskrat. Here the basic developments take place in a less complicated manner and show clearly the proper relationship of many features which it would be difficult to appreciate at first in a variable animal.

Color in non-variable fur-bearing mammals has been studied in detail by Hausman (1921), who, using muskrat hairs as a typical example of fur, has shown the histological basis for the variation in color throughout the hair-shaft, but this author failed to notice the marked difference in the distribution of the pigment in the proximal portion of the hair-shaft, depending upon whether the hair is taken from a prime or unprime area of the skin.

In hairs taken from an unprime pelt the pigment is continued down the hair-shaft into the root, and the condition of unprimeness in pelts may be shown to be due, not to pigmentation of the dermis or leather, but to the massed effect of the pigmented hair-roots. Conversely, primeness is due to the blanching of the hair roots; this process, however, does not terminate at the level of the epidermis,

but is continued out into the hair-shaft to a variable distance in different animals, and has been used by the author (1932) as the basis of a test for the detection of primeness in the pelts of *living* fur-bearing animals.

The present paper attempts: (1) To show the exact extent of variation in the priming process in a large number of fur-bearing animals, and to discuss its correlation with the nature and classification of these mammals; (2) to demonstrate the existing relationships between life cycle of the fur-hairs and the sequence of the blanching, priming and moulting processes.

The extent of blanching beyond the epidermis has been measured in the following animals, which include examples from three orders of mammals:

(1) Carnivora   (2) Otter (*Lutra canadensis*) Kuhl
                 (3) Silver fox (*Vulpes fulva*) Desmarest
                 (4) Mink (*Putorius vison*) Schreber
                 (5) Lynx (*Lynx canadensis*) Kerr
                 (7) Jaguarondi cat (*Felis cacomitili*) Baird
                 (8) Cross fox (*Vulpes fulva*) Desmarest
                 (11) Fitch (*Mustela putorius*) Boitard
                 (12) Civet cat (*Arctigalidia fusca*)
                 (13) Red fox (*Vulpes fulva*) Desmarest
                 (15) Weasel (*Putorius noveboracensis*) Emmons
                 (18) Arctic white fox (*Vulpes lagopus*)
(2) Rodentia   (1) Muskrat (*Fiber zibethicus*) Link
                 (6) Black rabbit (*Lepus cuniculus*)
                 (9) Grey squirrel (*Sciurus arizonensis*) Coues
                 (10) Beaver (*Castor canadensis*) Kuhl
                 (16) Varying hare (*Lepus americanus*) Erx
                 (17) Jack-rabbit (*Lepus campestris*) Bach
(3) Marsupialia (14) Common opossum (*Didelpys virginiana*) Kerr

Samples of under-fur hairs were obtained from prime pelts of the above-named animals by shaving them off close to the skin. Fig. 1 shows the relative degrees of depigmentation in the fur of these animals.

From a study of the figure it is evident that the extent of depigmentation is not related to the zoological classification, since the Rodents and Carnivores are found at both extremities. Again, there is no definite relation

between the extent of blanching and the length of fur, nor is there any corresponding proportion between the extent of this process and the width of the hairs, as Hausman (1924) has been able to demonstrate in the case of the ''Scale Index.''

Examination of the silver, cross and red fox hairs, which eliminates any chance of variation due to difference of species, suggests that density of pigmentation is the determining factor and also that this is a genetic character and is transmitted as such, for measurements show that the cross fox is situated midway between the silver and red fox, of which it is a hybrid.

There is a definite preponderance of semi-aquatic animals at the lower end of the chart, while the variable animals at the other extreme are all land mammals living in the northern hemisphere. Another interesting observation is that fur-bearers show a wide variation in the season at which the different mammals became prime. The pelt of the rabbit (*Lepus americanus*) is prime during December and January, while that of the muskrat at the other extreme becomes prime late in the spring, toward the end of March or in April.

It may be suggested that the variation in the time of priming can be explained upon the basis of the different mode of living found in these groups of animals. The former or semi-aquatic group is protected from the elements until spring, when its members are flooded out of their burrows, etc., while in the case of the land animals they are exposed to the sudden changes of temperature early in the winter season.

Examination of Fig. 1 shows the progression of depigmentation in a black, a snowshoe, and a jack-rabbit, lending support to the view that density of pigmentation is probably the major factor determining the amount of blanching in a given hair-shaft. It can be seen that the snowshoe rabbit occupies an intermediate position in showing the development of this feature, since the blanch-
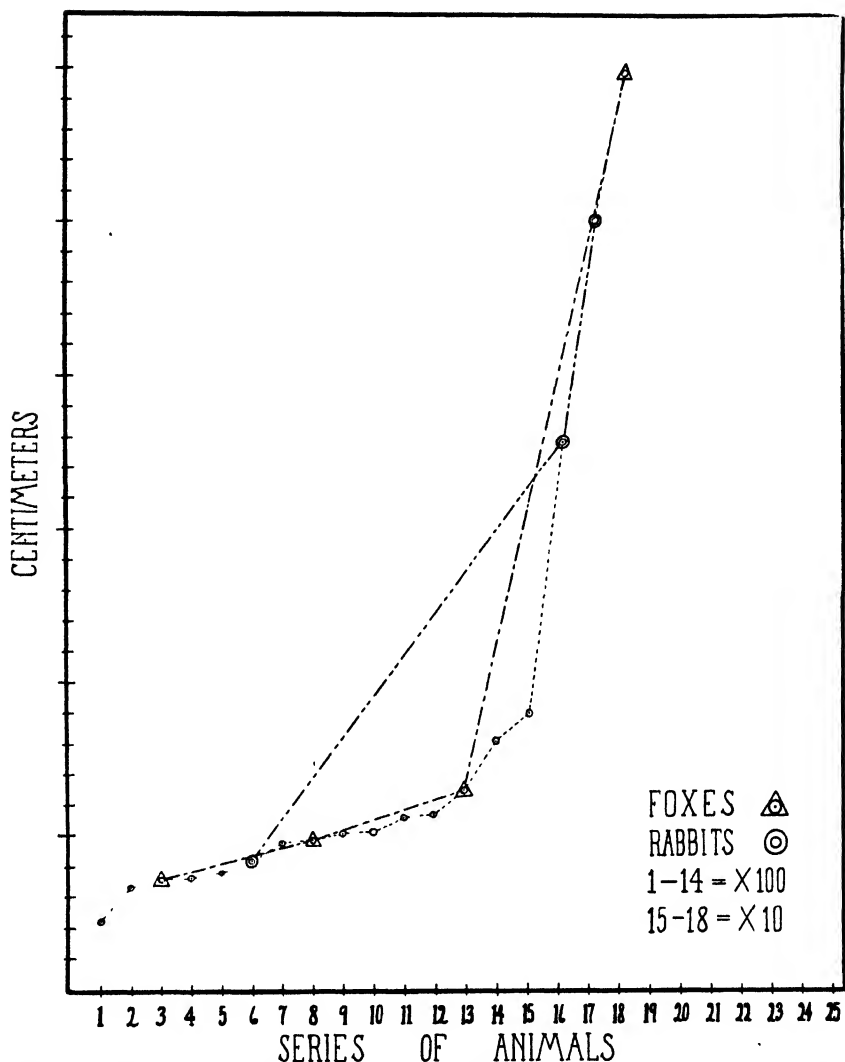
FIG. 1. Shows the relative amounts of depigmentation in the prime under-fur-hairs of a series of mammals (Key in text).

ing process takes place in such a manner as to leave the middle portion of the hair-shaft the last to become white. Reference to Fig. 2, III shows the intermediate state in the depigmentation process, corresponding to the winter pelage of the rabbit (*L. americanus*).   Hence this animal does not show the priming process to as great an extent as the jack rabbit, etc., in which the process does not stop until the fur-hairs are completely blanched (Fig. 2, IV).

The seasonable color change therefore in variable animals may be attributed to an exaggeration of the priming process, which takes place annually in all other fur-bearers, to a variable but lesser extent, save in albinos.

Further evidence in support of this view is seen in the fact that the presence of the summer or pigmented coat in variable animals is coincident with the unprime state, while the blanched or winter pelage accompanies the prime condition of non-variable mammals.

Again, the sequence of blanching and the priming processes are the same—the mid-dorsal area is not only the last part of the skin to assume the winter coloration, but is also the last portion of a muskrat pelt to become prime.

Hadwen states with regard to the rabbit (*L. americanus*) :

Probably the most convincing proof that the change takes place in existing hairs is to be found in the skin itself, when the hair roots are examined. The fact that the roots cease to function as the hairs turn white, and that it is a progressive change, offers conclusive evidence that the alteration is destructive.

Again, in the same paper, with regard to the Arctic white fox, this author states:

Portions of unprime white fox skins taken early in the winter show colour changes very similar to rabbits. Degenerating hair roots are found almost indistinguishable from those of a rabbit. It is evident that the alteration of color in the fur is preceded by loss of function on the part of the hair-roots.

We can not agree with this interpretation of the facts, however, that the blanching is due to the so-called degeneration of the hair-roots, since this process takes place in the roots of all fur-bearing animals which do not change color when they reach the prime state. Again, the change which takes place in the hair-roots, described in a previous paper (Gunn, in press), can not be looked upon as a destructive, but rather as a mature phase in the life cycle of the hair (Fig. 2, IV). The hair-roots

must reach this state before a pelt becomes prime, and it is well recognized that the length, density, sheen, texture and color of a pelage are seen in the optimum state only when the prime condition is reached. The nature of the priming and blanching process are therefore the same, the only difference being one of degree.

The relationship between a ripening process and the assumption of primeness is more clearly seen when the life cycle of the hair is studied.
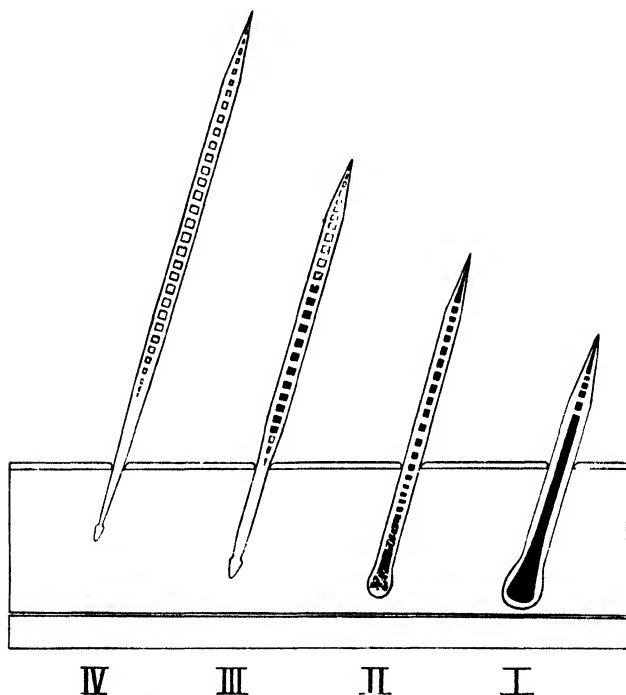
FIG. 2. Diagram showing four stages in the life cycle of an under-fur-hair of a variable mammal such as the jack-rabbit or Arctic white fox.

The different phases in the life of a fur-hair are shown in the diagram (Fig. 2). I represents a young hair which is densely pigmented down into the root. The massed effect of such hairs causes the pigmented condition seen on the fleshy side of an unprime pelt.

II represents a hair of the summer pelage in a variable animal, in which the melanin is not so densely congested in the root, and the hair has increased in length.

III shows a hair blanching, the root and tips preceding the intermediate portion of its shaft.

IV represents the totally blanched hair, typical of the winter pelage of the jack-rabbit, weasel and Arctic white fox, which has now reached its maximum length (the lengthening process referred to by Merriam, Welch, etc.) and may be considered as mature or prime. The mature phase lasts a definite length of time, and then this hair is shed. If it is not pulled out as probably is the case in the wild state, the old hair is pushed out upon the apex of the young hair growing beneath it and is eventually brushed off. It is now clear that the sequence of the growth of new fur, of the priming condition and of the moult is the same, and that the growth of new fur and the process of moulting proceed synchronously, but the prime phase is separated from these by a definite period of time. That different phases of the life cycle of the hair are present simultaneously during most of the year is seen in the prime and the unprime chart (Fig. 3).

This chart was constructed from the facts ascertained by studying a large number of muskrat pelts taken at different seasons of the year. Each concentric circle represents a cross-section of a pelt during a given season of the year:

I represents an early summer pelt in which only the ventral portion is unprime.

II is a mid-summer pelt, in which the ventral and lateral regions are unprime.

III represents a late summer pelt, the unprime condition prevailing throughout. Here the exact converse of the prime condition is seen.

IV is a fall pelt, in which only the ventral region is prime.

V represents the winter conditions of the pelt in which the ventral and lateral regions are prime, and the mid-dorsal area is unprime. ·

VI represents a spring pelt (the prime season of the muskrat) totally prime or devoid of pigmentation.

DORSAL
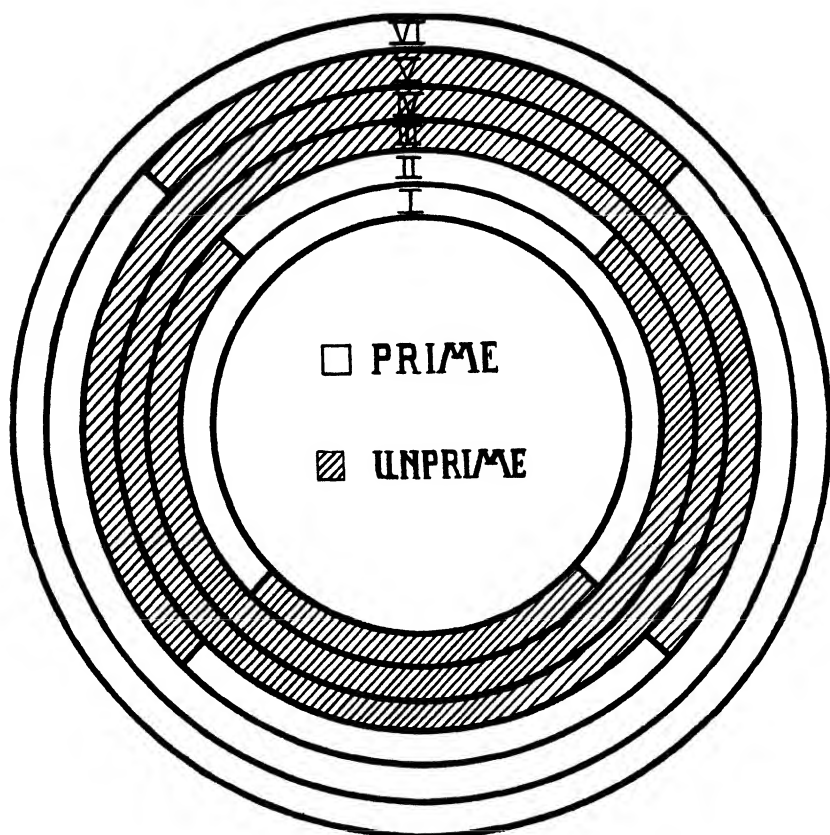
VENTRAL

Fig. 3. Prime and Unprime Chart. The concentric circles represent trans-
verse sections of pelts at different seasons of the year and show
the sequence of the priming process.

This chart is also applicable to the rabbit, etc., if the
seasons are adjusted, *e.g.*, the rabbit (totally prime dur-
ing December) becomes prime earlier than the muskrat
(totally prime during April), and likewise the other
phases are seen proportionally earlier in the year. Re-
ferring to the chart it is evident that the different phases
of the life cycle of the fur hairs are coexistent over the

body surface, except in the state of total unprimeness (III) and total primeness (VI), but it has been shown that where new fur is growing some shedding is present; hence this process does not take place simultaneously over the different body surfaces, but follows a definite sequence and is therefore present in a lesser degree throughout the year, save when the animal is totally prime. Moulting is at its height, however, when the pelt is totally unprime in the spring (III).

This explains the difficulty Hadwen (1929) encountered with regard to this condition, when he states,

At no time during the year has the writer seen a heavy moult, such as one finds in tame animals, nor any evidence of matting as it occurs in long-

FIG. 4.   Shows five stages in the change from summer to winter pelage in weasel pelts.

haired tame rabbits. Undoubtedly the greatest amount of hair is lost in spring, and a certain amount of the coat is loose and ready to fall out not long before the autumn change of color. Even after the hair has become white it is possible to pull out dead hairs which have all the appearance of belonging to the spring coat, they are black at the tip and have a light yellow zone. It is probable that these are the parti-colored hairs referred to by Allen. However, though some shedding goes on during the change, it represents only a very small percentage of the total hair covering. It never seemed probable that the rabbits would shed all their hair in the autumn and replace it during the early part of winter when they are in need of warmth. Furthermore it seems an impossibility for a pigmented animal like a rabbit to grow white hair unless it is an albino.

The lesser amount of moulting noted by Hadwen during the season when the coat was partly unprime is also objected to by fur-dressers when dressing unprime muskrat, beaver pelts, etc., (Gunn, 1932) and has been shown to be due to a remnant of the old coat in an unprime area of skin.

Hadwen (1929), Welsh (1869), Allen (1894), etc., have noted that the change in color follows certain areas with great regularity, as is also seen in the weasel (Plate I), but unfortunately they were unable to follow the sequence of the moult by means of the color change, owing to the fact that the rabbit remains white on its ventral surface throughout the year. The ventral region, however, becomes prime first and moults first. Not only is there definite evidence of this due to visible shedding in the ventral region, but if the fleshy side of the pelt be examined about February 1st, it is seen to be pigmented due to the growth of young hairs in this region. In other words, the cycle commences here again and is accompanied by moulting on this same surface of the body.

From a further study of the chart (Fig. 3) it would be expected that the period of total primeness would be of short duration, which agrees with the facts, for it is found that total primeness only lasts approximately one month (December in *L. amer.*). This is explicable, since the total condition only lasts from the time the dorsal surface becomes prime until the ventral surface shows

pigmentation again. The ventral areas become prime first and remain so while the sides and back reach this state in their turn. Hence, the dorsal surface is not prime long before the ventral region begins the cycle over again.

It has been suggested that the sequence followed by the priming process is due to the fact that the ground becomes cold in autumn before the sun loses its intensity, and therefore the maximum growth of fur is first required on the ventral surface. The same reasoning probably applies to the color change in variable animals, namely, that it is a modification of a phenomenon common to all fur-bearing animals, brought about through long inheritance, resulting in a better adaptation to environment, whether it be from protective coloration, from resistance to the rigors of winter or the heat and actinic rays of summer.

In summarizing it is evident:

(1) That the prime or mature condition of the fur occurs annually in all non-variable mammals (save albinos) and is coincident with the optimum attributes of the fur and with depigmentation of the hair-roots and shaft to a variable extent in different animals, and that autumnal color change is due to blanching of the summer coat or is merely an exaggeration of the same condition.

(2) That the pigmented summer coat of variable mammals corresponds to the unprime state and conversely the winter pelage of variable animals is comparable to the prime condition of non-variable animals.

(3) That the change from winter to summer coat in the jack rabbit, weasel and Arctic white fox is due to shedding of the white winter pelage and the growth of pigmented summer fur.

(4) That the same sequence is followed in the priming and blanching processes, in the moult, in the growth of new fur and therefore in the pigmentation of the pelt.

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SHORTER ARTICLES AND DISCUSSION

THE DISTRIBUTION OF GENES AMONG ISOMETRIC CHROMOSOMES

IN a number of species, particularly of plants, the chromosomes of a set display no appreciable, or at least no marked, differences in size and form; the chromosomes are practically isometric and isomorphic. The 12 pairs of the tomato, for instance, are "short rods showing no distinct individuality" (Lesley, 1), a statement that is confirmed by the descriptions and illustrations of Winkler and other investigators. Vilmorin and Simonet (2) say, "la grosseur et la forme des chromosomes dans les celles mères des grains de pollen sont assez homogènes," and Jørgensen (3) found, in both somatic and meiotic divisions, that "the chromosomes are, as far as can be ascertained, identical in size and shape." A similar striking homogeneity is especially mentioned and clearly depicted as almost a general characteristic of the species of *Solanum* (2, 3), *Physalis, Capsicum, Atropa, Petunia, Salpiglossis, Lobelia, Linum* and *Campanula* (2). The chromosomes of *Primula sinensis* are "of very even size" (Sömme, 4). Winge (5) remarked that, in the sweet pea, "there is no difference, on the whole, in the size of the chromosomes," and that in *Antirrhinum majus*, another form of genetic interest, there are "eight chromosomes of uniform size." Such isomorphism is noted or shown by various authors in at least some species of *Aquilegia, Pentstemon, Godetia, Eschscholtzia, Rosa, Raphanus, Brassica* and *Vitis;* and a special search would undoubtedly find it wide-spread and common. In not a few insects the chromosomes are all "of practically the same size and shape" (6). Even in the fish, *Lebistes,* with 23 pairs, including an XY pair, "the chromosomes are very much alike" (7). It seems probable that, because of the interest attaching to individuality of chromosomes, especially of sex chromosomes, the species showing marked heterogeneity have been singled out and emphasized more than their numbers alone would justify. But pointing out the existence of these nearly isometric sets is not to be construed as denying or contradicting any fundamental tenet of the chromosome theory; the component chromosomes of such sets presumably possess as distinct a qualitative individuality as do those, for instance, in *Zea, Datura, Iris, Nicotiana,*

Drosophila or mammals, which are also more or less obviously quantitatively differentiated in size and form.

While the familiar studies with *Drosophila,* the pea, sweet pea, maize, snapdragon and *Datura* are general guides to the methods to be used and the results to be expected in linkage and chromosome works, it would seem that additional aids and guides might be developed for the geneticist and breeder undertaking a factorial analysis of those species, where size individuality in the complements is essentially lacking. Such analyses are likely to be made often in the future to test further the chromosome theory, to build up the objective data of a comparative genetics, and, in the applied field, to provide a foundation of knowledge for the use of the practical breeder.

In a genic analysis, the first gene studied will fall to some one chromosome, leaving the others unoccupied by known genes. As further factors are located, the hitherto unoccupied chromosomes will one by one acquire markers and gradually fill up with linked genes. At any stage in this process, it would often be interesting and helpful to know, and it is possible to predict for the species possessing chromosomes of approximately equal size: (1) (a) the proportion of chromosomes occupied or unoccupied by known loci; (b) the frequency of chromosomes carrying 1, 2, 3, 4 or more genes each; (c) the approximate number of genes it will be necessary to study to place at least one in each chromosome; (2) the proportion of factors that occur singly, or linked by twos, threes, etc.; and (3) the proportion of dihybrid combinations showing independent assortment, or linkage.

Theoretical Distribution of Genes among Isometric Chromosomes

Chromosomes of equal size (length) may be considered as approximately equal in gene content or number of loci, and for statistical purposes may be regarded as equal members of a larger group, the genom. The expected or probable distribution of genes, taken wholly at random, in such a set should obey certain statistical rules.

The theory involved is really that required to find the expected distribution of m objects (genes) in n boxes (haploid chromosomes), all of equal size and accessibility (9). This distribution is derived from the binomial, $(q + p)^m$, or $\left(\dfrac{n-1}{n} + \dfrac{1}{n}\right)^m$, where m

is the number of genes for which satisfactory linkage data are available, n is the haploid chromosome number, and p and q the chances of success or failure of any gene studied being in any one particular pair of chromosomes. That is, $p = \dfrac{1}{n}$ and $q = \dfrac{n-1}{n}$, and $p + q = np = 1$. As n increases from 1, 2, 3 to 7 and 12, q rises from 0, $\frac{1}{2}$, $\frac{2}{3}$ to $\frac{6}{7}$ and $\frac{11}{12}$, and p falls from 1, $\frac{1}{2}$, $\frac{1}{3}$ to $\frac{1}{7}$ and $\frac{1}{12}$. When n is 2, then $p = q$, and the frequency distribution is symmetrical, but when n is 3 or more, q exceeds p, and the distribution is asymmetrical. Since the observed haploid chromosome number is commonly 6 to 12 or more, the initial asymmetry is characteristically very marked; when n becomes very large the law of small chances would apply (9).

The theory and derivation of this binomial and the most convenient methods of calculating its terms by logarithms have been described by Yule (9) and Johannsen (8). For valued helps and pertinent criticisms the writer is indebted also to Messrs. I. R. Pounder, C. P. Winsor and Sewall Wright.

<div align="center">APPLICATIONS TO LINKAGE STUDIES</div>

(1) *The expected proportions of chromosomes occupied or unoccupied.* If we observed a great number of cases in which a series of m distinguishable genes were distributed at random, either simultaneously or consecutively, among n chromosomes of equal size, what would be the relative frequency of chromosomes containing 0, 1, 2, 3, 4 . . . m genes?

This distribution would be proportional to the terms of the binomial, $\left(\dfrac{n-1}{n} + \dfrac{1}{n}\right)^m$, and the probabilities of chromosomes being unoccupied, or occupied by 1, 2, 3, 4 . . . m genes are indicated by the numerical frequencies of the successive terms:

$$\left(\frac{n-1}{n}\right)^m + m \cdot \left(\frac{n-1}{n}\right)^{m-1} \cdot \frac{1}{n} + \frac{m(m-1)}{2} \cdot \left(\frac{n-1}{n}\right)^{m-2} \cdot \left(\frac{1}{n}\right)^2 + \frac{m(m-1)(m-2)}{2 \cdot 3} \cdot$$
$$\left(\frac{n-1}{n}\right)^{m-3} \cdot \left(\frac{1}{n}\right)^3 \ldots \left(\frac{1}{n}\right)^m$$

The full array of $m + 1$ terms occurs with a minimum of $(n)^m$ distributions.

The same expectancies may be calculated by permutation methods. In general, if we ask the probability that a particular chromosome shall be found to contain just k out of the m genes,

this probability (corresponding to the relative frequency of any term of the binomial) may be calculated from the formula:

$$\frac{m!}{k!\,(m-k)!} \cdot \left(\frac{1}{n}\right)^k \cdot \left(\frac{n-1}{n}\right)^{m-k}$$

From the basic binomial the probability of a chromosome being occupied by at least one gene is $1 - \left(\dfrac{n-1}{n}\right)^m$, and the mean number of occupied chromosomes is n times this value, or $n - \dfrac{(n-1)^m}{(n)^{m-1}}$. To be reasonably certain that markers will be found for all the chromosomes, the number of factors identified and placed must be such that $\dfrac{1}{n}$ well exceeds $\left(\dfrac{n-1}{n}\right)^m$; the expectation that no chromosome of the whole set shall be unoccupied by some one of the genes evidently can not be stated precisely in a simple way, but will lie between the not very wide limits, $1 - \left(\dfrac{n-1}{n}\right)^m$ and $1 - \dfrac{(n-1)^m}{(n)^{m-1}}$.

(2) *Distribution of the genes.* As regards the factors themselves, their mean number per chromosome is $\dfrac{m}{n}$, but they may occur singly or linked. The expected proportion of single markers and of genes linked by twos, threes, fours, etc., may be determined directly from the terms of $\left(\dfrac{n-1}{n} + \dfrac{1}{n}\right)^{m-1}$.

(3) *Proportion of dihybrid combinations showing linkage.* The probability that a pair of genes will be linked is clearly $\dfrac{1}{n}$. The total possible number of different pairings of m genes is $\dfrac{m(m-1)}{2}$. Combinations involving genes from different chromosomes assort freely, and those involving genes of the same chromosome should show linkage. The ratio of intra-chromosomal to the total possible number of combinations may be derived either from the binomial distribution or directly, the expected number of pairs showing linkage being $\dfrac{m(m-1)}{2} \cdot \dfrac{1}{n}$. The failure of genes far apart on a chromosome to show definite linkage tends to lower this proportion, but the practice of making linkage test combinations with only a few appropriately placed markers tends to raise the proportion greatly.

AGREEMENT BETWEEN OBSERVED AND THEORETICAL
DISTRIBUTIONS

In the tomato, where n 12, and m represents the first 18 qualitative genes identified and studied in nearly all their possible dihybrid groupings, it now appears that one chromosome carries 4, one 3, three 2, five 1 and two none of these genes. These may be compared with the theoretical random groupings derived from $(\frac{11}{12}+\frac{1}{12})^{18}$ (Table 1).

TABLE 1

SHOWING THE CORRESPONDENCE BETWEEN EXPECTED AND OBSERVED DISTRIBUTIONS OF GENES IN THE TOMATO, PRIMULA AND SWEET PEA

		Frequency of chromosomes carrying							X^2	N = n-1	P
		0 gene	1 gene	2 genes	3 genes	4 genes	5 genes	6 genes			
Tomato											
expected	$(\frac{11}{12}+\frac{1}{12})^{18}$	2.5	4.1	3.2	1.5	0.5	0.2		10.	11	.53
observed	..	2.	5.	3.	1.	1.	0.				
Primula											
expected	$(\frac{11}{12}+\frac{1}{12})^{10}$	5.0	4.6	1.9	0.4	0.1			7.6	11	.75
observed[4]		6.	4.	1.	0.	1.					
Sweet pea											
expected	$(\frac{6}{7}+\frac{1}{7})^{19}$..	0.4	1.2	1.8	1.7	1.1	0.6	0.2	3.1	6	.80
observed[11]		0.	2.	0.	4.	0.	1.	0.			

Of the 18 factors in the tomato 13 have been found linked and 5 single, where the probabilities on a random distribution were 13.9 and 4.1, respectively. Of the dihybrid combinations 7.8 per cent. were found linked, where 8.3 per cent. were anticipated. In general, the observed distribution appears by inspection to be consistent with the assumption of their random allotment among the approximately identical chromosomes. As a measure of the goodness of fit, should the conditions warrant so exact a procedure, the Chi-square test may be applied, when the number of genes is fairly large, since all equally probable combinations will yield the same X^2. Deviations are taken from the expected number of genes per chromosome, $\frac{m}{n}$. Thus the probability is .53 of getting a worse system of deviation than was observed in the tomato.

It would be interesting to apply similar tests to other cases where the basic conditions are reasonably well met. The chief difficulties encountered lie in the incompleteness of the objective data—not all the factor combinations having been tested, or the number of linked groups exceeding n, as in the pea (10)—and in the strong inclination of workers to select for first study genes which promise to belong to large linkage groups. The observations in Lathyrus (11), Primula (4) and possibly in Pisum (10) do not indicate any very significant departures from a chance distribution.

SUMMARY

For the rather numerous species, whose chromosomes exhibit little or no size differentiation, it is suggested that some guidance in a genetic analysis may be found in calculating the theoretical distribution of groupings of m genes among the n pairs of chromosomes, by adaptations of the binomial formula, $\left(\dfrac{n-1}{n} + \dfrac{1}{n}\right)^{m}$. From these the geneticist may derive the expected proportion of chromosomes carrying 0, 1, 2, 3, 4 . . . m genes each; the approximate number of genes required to place at least one in each chromosome; the proportion of single and of linked genes; and the proportion of dihybrid combinations that will assort freely, or show linkage.

In the tomato the observed groupings of the first 18 factors identified and located seem consistent with the assumption of their random distribution among the 12 pairs of approximately equal chromosomes. The distribution of known loci in *Primula sinensis* and the sweet pea also accord reasonably well with expectation.

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AN EVALUATION OF SIZE GENES

In a previous communication to this journal the author (1931) reported an association in heredity between size and coat color in mice. The data were derived from the back-cross generation of a cross between a strain of large *Mus musculus* carrying the recessive genes for dilution, brown and non-agouti and a race of small *Mus bactrianus* possessing the corresponding dominant allelomorphs. When the F_1 animals were mated to the recessive parent race, eight color classes of approximately equal numbers were obtained. A comparison of the dominant and recessive members of the three factor pairs involved disclosed an indubitable association between certain size and color characters. The size characters investigated included skull length, skull width (interorbital width), humerus, femur and tibia lengths, adult weight, body length, tail length and cranial capacity. Of these, the greater weight, humerus, femur and tibia lengths and body length, characteristic of the recessive *musculus* parent, were found associated with brown coat color derived from the same parent, while greater body and tail lengths likewise tended to be characteristic of those animals exhibiting the recessive gene for dilution. In those instances in which the mean difference between recessives and dominants

(brown, black and dilution, intensity) was as great as or greater than four times its probable error, genetic linkage between qualitative and quantitative characters was considered as demonstrated. It was not held that humerus length, for example, was determined entirely by a gene or genes linked with the gene for brown, but merely that such genes were partially determinative.

Although an interpretation making use of genetic linkage appears to fit the data satisfactorily, Castle (1932) has offered an alternative "non-chromosomal" explanation, which, however, it seems to me, is less tenable.

Since it was realized that the quantitative genes linked with b, for example, were responsible only in part for the respective size characters, an attempt to evaluate the size genes, to determine approximately the portion of the variability in the quantitative characters brought about by genes linked with the qualitative characters concerned, seemed advisable. I am indebted to Professor Sewall Wright for kindly suggesting the following method of analyzing the variance directly.

In general, the formula for the standard deviation due to b, B, for example, is $\sqrt{q(1-q)D^2}$, where q is the proportion in one class (browns), 1–q, the proportion in the other class (blacks) and D the difference between the means. The ratio of the variance, $q(1-q)D^2$, to the variance of the total (mixed) population (σ^2) gives the correct degree of determination except that no allowance is made for sampling differences. With numbers as large as ours, however, this can be ignored with the loss of practically little of significance.

Determination of Variance by Genes Linked with b, B

Character	Sex	No. of mice	Degree of determination
Humerus length	♂	152	12.56 per cent.
	♀	139	8.58 " "
Femur length . .	♂	151	9.25 " "
	♀	138	8.48 " "
Tibia length	♂	152	4.85 " "
	♀	139	6.56 " "
181st day weight . .	♂	153	8.33 " "
	♀	140	9.68 " "
Body length	♂	153	5.50 " "
	♀	140	5.48 " "

Determination of Variance by Genes Linked with d, D

Character	Sex	No. of mice	Degree of determination
Body length	♂	153	4.71 per cent.
	♀	140	4.71 " "
Tail length	♂	149	10.64 " "

A consideration of the cases in which linkage was observed, *i.e.*, cases in which the difference between the means of the recessive and dominant members of the factor pairs was as great as or greater than four times its probable error, gives the following results:

Of course the actual size factors may be responsible for a larger portion of the variance if not completely linked with the qualitative gene.

The above computations indicate that the size genes definitely located account for a distinct, although perhaps rather minor, portion of the variance in the respective size characters. It further appears probable that the latter are influenced as well by several or many genes situated in a number of chromosomes.

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THE INCIDENCE OF MAMMARY CANCER IN A CROSS BETWEEN TWO STRAINS OF MICE

The report to be presented deals with a cross between a non-yellow (dilute brown) strain of mice high in the incidence of spontaneous carcinoma of the breast and a line of yellow mice lower in cancer incidence. In the latter line the type of cancer is usually sarcoma of various sorts. Data will be offered in a later paper, dealing more fully with the tumor-forming characteristics of both parent strains.

The object of the present report is to establish the existence of a clear difference in cancer incidence between the yellow and the non-yellow F_2 hybrids descended from the above cross.

It has long been recognized that most yellow mice have a type of general metabolism which differs from that of most non-yellows. This results in distinctly greater adiposity in yellow animals. The opportunity to contrast the incidence of mammary cancer in yellow F_2s and in their non-yellow sisters seemed therefore to be of interest.

In the cross to be recorded, virgin females were used in F_2 to determine the carcinoma incidence. This was done for two reasons. Females were chosen because the incidence of carcinoma of the breast in the stocks chosen is confined to that sex. Virgins were selected because there is evidence from the work of several investigators that the exercise of the reproductive function is a factor of great variability and complexity in contributing to the etiology of carcinoma of the breast in mice.

In the stocks used there is clear evidence that adenocarcinomas of the breast may appear as advanced stages in the development of adenomas. These more or less benign tumors are therefore included among the cancer class in the data presented.

There was a total of 260 F_2 virgin females which lived to an age sufficient to allow them to be included as critical data in determining cancer incidence. Of these 136 were yellow and 134 non-yellow. Among the two classes the incidence of cancer of the breast was as follows:

	Cancer	Non-cancer	Per cent. cancerous
Yellows	53	83	38.97 ± 2.81
Non-yellows	80	54	59.70 ± 2.85

The difference (20.73 ± 4.00) is 5.18 times its probable error. There is no question, therefore, that a significantly higher proportion of non-yellows than of yellows form cancer of the breast in this particular hybrid generation.

The next matter of interest is to attempt to obtain some information as to the cause of the difference.

The two explanations which seem to be the most probable are (1) that genetic linkage exists between a gene determining high incidence of mammary cancer and the gene for the non-agouti

(a) allelomorph of yellow (A^y), and (2) that there is something in the actual metabolism of a yellow (A^y) mouse which makes that animal unfavorable to the development of mammary carcinoma.

Data are not at present available to decide finally between the two possibilities. Certain evidence bearing on them may, however, be mentioned.

If the physiological explanation is the correct one it might easily follow that yellow mice would have a different life span and so might not be strictly comparable to non-yellows. Comparison in respect to this factor, however, shows that no significant difference exists in this respect between yellows and non-yellows as a whole. If, however, yellow cancerous mice are contrasted with non-yellow cancerous animals it is found that the mean age at death of the yellows is 476.0 ± 14.26 days, while that of the non-yellows is 550 ± 13.62 days. This difference is 3.7 times its probable error. If the difference is significant, as it may well be, it becomes important to compare also the age at death of non-cancerous yellows and non-cancerous non-yellows. When this is done the mean age of the yellows is 599 ± 11.48 days and for the non-yellows 628 ± 13.00 days. The difference is only 1.6 times its probable error and is not significant.

It is clear, therefore, that the evidence from these data shows no physiological handicap as such to the formation of cancer of the breast in the yellow mice. Provided an individual yellow mouse is going to have cancer it actually dies of it significantly earlier than do the non-yellows. This signifies for the yellows either earlier incidence of cancer or greater malignancy or both. Either or both these facts tend to militate against the probability of the existence of any general metabolic factor discouraging to cancer formation and peculiar to yellow animals.

A certain amount of supplementary evidence as to the comparative malignancy of tumors of the breast in yellow and in non-yellow hybrid mice is also available. Since adenomas are of a lower grade of malignancy than carcinomas a tabulation of the comparative incidence of this type of neoplasm and of carcinomas in yellows and in non-yellows can be made. If the yellows have a general metabolic factor which decreases the likelihood of malignancy, these figures should show it. Actually of the 53 tumors in yellows 44, or 83 per cent., are carcinomas, while 17 per cent. are adenomas. Of the 80 tumors in non-yellows 57,

or 70.4 per cent., are carcinomas and 29.6 per cent. are adenomas. The figures certainly show no decrease and possibly an increase in malignancy in the tumors of yellow animals. This evidence suggests further the improbability that a general metabolic factor is involved.

With these facts in mind we may conclude that a careful breeding experiment, using agouti (A) rather than yellow mice (A^y) as the low cancer strain, should be carried out. This should suffice to show whether linkage between high incidence of cancer of the breast and the non-agouti (a) locus exists.

Whether it does or not, a difference in incidence of mammary cancer between yellows and non-yellows is already clearly established and forms the first evidence of an important interrelationship between a color variety of mice and the spontaneous incidence of mammary cancer.

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RECOMBINATION AND CROSSING-OVER

The interpretation of the term "percentage of recombination" as "percentage of new combinations" (Hutt, Am. Nat., 66: 274) is not only entirely correct, it is orthodox as well. "Recombination" has been consistently used as synonymous with "new combination" throughout; for example, in the explanation of Drosophila symbolism given by Bridges and Morgan (Carnegie Pub. No. 327, p. 9).

When linked genes enter a cross certain individuals in the second generation may show new combinations of the characters that entered from the different parents. Thus, if Dichaete is crossed to pink, and the F$_1$ female is back-crossed to a pink male, most of the flies are of the two original types, Diachaete or pink; but a small number of the offspring are both Dichaete and pink or neither (*i.e.*, wild type). These two latter classes are called "recombination classes" and the "percentage of recombination" may be found by dividing the sum of the recombination classes by the total number, and multiplying this decimal fraction by 100. . . . The use of the term "recombination" in this technical sense is a shortening of the full term "recombination of linked characters."

But while Hutt agrees with this synonymy of new combinations with recombinations, as this term has been defined and used, he is of the opinion that on etymological grounds this meaning, in any sense of the word recombinations, is not legiti-

mate; that in the true interpretation this term is applicable only to the genotypes and phenotypes which are the same as those of either parent. It sometimes happens that there is more than one well-defined true meaning of a prefix, and "re-" is a good example of this. According to the Century Dictionary, "re-" has, besides its meaning of "back" (illustrated in such words as remit, reflex), a meaning of "against" (react, rebel, resist). Besides the meaning "restoration to a former state" (reintegrate, reset), it has the meaning "transition to an opposite state" (revolution, recant, reform). The meaning "repetition of a former act" is evident in the words rearrange, redeal, redye, remarry, reword, rewrite. It should be noted that it is the act which is repeated, while the objects acted upon or with may be entirely new (as in remarry); or the result of the action upon old objects may be something new (as in rearrangement, redeal, redye). The word "replace" means either restoration to a former state (to replace a key upon its hook) or transition to a new state (to replace a hydrogen by a carboxyl). While "recombine" is not exhaustively defined, its close analogy to "rearrange" (to arrange anew, make a different arrangement) and the thoroughly accredited use of "re-," with the connotation of transformation to a new state or result, make it entirely permissible for "recombination" to be employed to designate new or different combinations, especially if its use is in a technical field and is prefaced by a clear statement of its intended significance.

There is an objection to be raised from another point of view, namely, that "recombination" had been already used in genetics, in connection with the $9:3:3:1$ ratio of non-linked characters. This priority was of course known, but in the early days when recombination was used in connection with the F_2 ratios, the emphasis was entirely upon the end result, namely, the classes of individuals, and the distinction as to the recombinations being produced by reassortment or by crossing-over had not arisen. In the definition of "recombination" by Bridges and Morgan, quoted above, it is recognized that the recombination of linked characters is a special use of the term instead of its general use, which did not specify the method of recombination as through reassortment rather than through crossing-over. That it was not restricted to non-linked characters in usage is illustrated by the other prior use of the word by Lotsy, where again the emphasis was upon the new forms which originate from the reshuffling and new combinations of the old genes.

Lotsy would not throw out of consideration as recombinations those cases in which the original two genes were carried by the same chromosome (hence recombination through crossing-over). Both of these early uses were *general* and did not exclude the *special* use of the term in connection with one or with the other mechanism of production of the recombinations.

As a matter of fact, the only cases where it would be uncertain as to whether the term recombination were being used in the one rather than in the other special sense are exactly those in which it isn't known whether the mechanism is reassortment or crossing-over, and hence the general sense is needed. Such cases are where the percentage of recombination is not a statistically significant departure from the 50 per cent. corresponding to random recombination.

One of the strong points in favor of the term "recombination" is exactly that it permits a clear separation of two categories, one the observed result (a series of classes of individuals) and the other a mechanism behind that seriation (reassortment or crossing-over). It was not recognized in the early days of linkage study that this distinction should be made and kept sharp. As a result it was almost impossible to be clear in the explanation of the relations of "map-distances" to the percentages observed in experiments. The "linkage values" or better the "crossover values" of the early papers meant indiscriminately now map-distances and now observed percentages. It could not be made a general law that "crossover values are additive" as long as "crossover values" could mean the "percentages of recombination of the characters." For example, the percentage of recombination for white and miniature was approximately 33, and for miniature and rudimentary approximately 18. If these be added, the result (51) is in excess of the value (42) observed for white and rudimentary. The common side-step was to say that the "apparent" crossover value was 42, while the "real" crossover value was 51. But as soon as one defines the observed percentage as a "percentage of recombination" and reserves the term "crossover value" for the map distances all becomes clear.

Whenever one uses the term crossing-over one refers now to the mechanism behind the recombination of the characters or of the genes for the characters. Crossing-over is something which occurs to the chromosomes at a particular point along their length. If one crossover falls *between* the loci for two pairs of characters then these characters will emerge as recombinations.

But the number of crossovers may exceed the number of recombinations per hundred gametes, for each double crossover leaves in their original alignment all loci outside the region between the two crossovers.

Now map distances and crossover values (synonymous) are always additive (aside from errors in experimental determination) while percentages of recombination are related through a complex formula involving the coincidence index for the special section considered. The increase in recombination percentage, as successively longer sections are considered, is not additive and approaches 50 per cent. as its limit. It becomes apparent that the simplest method of predicting the percentage of recombination corresponding to a given map distance, or, conversely, the map distance corresponding to an observed percentage of recombination, is through special correction curves based on all available information as to the specific situation for each specific region of each chromosome. Such a set of correction curves for the third chromosome is given on page 12 of the above quoted paper by Bridges and Morgan. Recombination is always expressed as a percentage, and map distance or crossover value never as a percentage but always as a number of units. This unit is carefully defined as that length of chromosome within which on the average one case of crossing-over occurs for each hundred gametes tested. The term "linkage value" should be dropped, since linkage is evaluated in crossover units, just as interference is now expressed in the index of coincidence.

The study of recombination percentages for linked characters is simply a means to the end of studying crossing-over frequencies and distributions. The center of interest is the crossing-over relations; hence it is emphasizing the symptomatic result rather than the underlying mechanism when one speaks of "recombination in fishes," rather than of "crossing-over in fishes." In his objection to this usage, Hutt is entirely right; but he is entirely wrong in supposing that the only term needed is crossing-over. Besides "percentage of recombination," the synonyms "percentage of new combinations" and "percentage of separation" have been used more or less in the Drosophila work. But the term "recombination" has the advantage over these other equally valid terms in compactness and in smoothness of its use in various situations by its ready transformation into the corresponding verb and adjective.

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